ERRATUM

Article 7147, Kramer and Schaeffer, on Experimental Poliomyelitis, last line of the table should read, " .5 cc. serums of 8 out of 9 monkeys neutralized, etc."

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7193 P*

Akee Poisoning.

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An illness on the island of Jamaica known locally as "vomiting sickness" is thought to be, at least in some cases, a form of poisoning caused by eating the fruit of the akee *(Blighia sapida)*. Although it is probably true that the name "vomiting sickness" has been applied to etiologically unrelated affections, there is strong evidence[1] implicating the akee in a serious and frequently fatal sickness. Deaths after eating akees are seemingly reported sometimes as vomiting sickness, sometimes as akee poisoning. The total deaths registered annually in recent years under these designations have run as high as 350 (1921). The akee in an immature or unripe condition has been reputed to be particularly dangerous.

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* P represents a preliminary, C a complete manuscript.

[1] Scott, H. H., *Ann. Trop. Med. and Parasit.*, 1916, **10**, 1.

Considerable unwillingness to accept these conclusions has been manifest, chiefly on the ground that the akee is a common market article in Kingston and elsewhere in Jamaica, and, in its season (November-March), is eaten daily by hundreds, probably by thousands, of persons with perfect impunity. It is also pointed out that with the improvement in water supplies that has taken place in several localities the "vomiting sickness" has greatly declined.

The edible portion of the akee is the arillus, a fleshy envelope attached to the seed. Arilli boiled or fried have been repeatedly eaten by one of us (E.O.J.) as by other Americans and European residents in Jamaica without any sign of discomfort or injury.

Through the kindness of Dr. B. E. Washburn, representative of the International Health Division of the Rockefeller Foundation in Jamaica, we have been able to secure a considerable number of akees in various stages of development. No signs of illness have been observed in experimental animals (cats and monkeys) fed with the arillus alone. The seeds, however, in both the ripe and unripe fruits contain a water-soluble poison which affects cats and monkeys, causing serious vomiting, and occasionally death when a large amount is given. The ovary wall or husk of the fruit also contains a toxic substance that is extracted with boiling water. When the whole fruit is chopped fine and extracted with boiling water, the extracts from both ripe and unripe fruits are poisonous.

The poisonous substance may be conveniently extracted by boiling the whole fruit, or the proper parts, in water for from 30 minutes to 2 hours. The seed extracts are composed, for the most part, of starch and a substance which hydrolyzes to a reducing sugar. The starch may be precipitated by the addition of alcohol. The poisonous substance remains in solution. The starch-free solution contains only a trace of reducing substances but gives a strongly positive Molisch test. After boiling in dilute hydrochloric acid solution the filtrate strongly reduces alkaline copper and picric acid. Ferric chloride and Millon's reaction are negative. Cyanide could not be detected. The evidence suggests that the poisonous substance accompanies a glucoside present in certain portions of the akee.

The explanation of the apparent epidemiological difference observed between the ripe and unripe fruit in Jamaica seems to be that in the latter the seed is small, soft and embedded in the arillus, while in the ripe fruit the large, dark brown, conspicuous seed has a hard shell and is easily removed from the edible portion. The placenta which attaches the arillus to the husk is also not readily removed from the unripe fruit, and is locally regarded in Jamaica as poisonous. We have not, however, been able to produce any illness in cats or a monkey by feeding them with placental extract. It is a reasonable explanation that in the country districts of Jamaica, where most of the reported cases of akee poisoning have occurred, akees that have fallen to the ground before ripening and those taken immature from the tree have not had the small seeds removed before eating. It is significant that many of the reported cases of akee poisoning have occurred in children, and that the "soup" made by boiling akees with various vegetables has a poisonous quality.

Although there can be no doubt that some of the cases of the vomiting sickness of Jamaica are caused by akee poisoning, the proportion of such cases in any particular time or locality can naturally not be determined without specific investigation.

Further experiments on the nature and action of the akee poison are in progress.

7194

Effect of Experimental Hyperparathyroidism on the Incisor of the Rat.*

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The microscopic anatomy of the incisors was studied in 2 groups of rats:

A. 29 rats that were given 1 to 15 injections of parathyroid hormone. Doses varied from 10 to 150 units. Weight: 85-145 gm.
B. 7 controls from same colony.

Significant alterations were observed only in Group A: 1. Enamel hypoplasia was found to be formed at the time of the first injection in 5 animals. 2. The alveolar bone showed an abnormal increase in osteoclasts in 14 and a fibrous change of the bone marrow in 3 of the animals that were given 3 or more injections of parathyroid hormone. 3. The principal changes were found in the dentin. Each experimental animal showed a primary hypocalcified stripe in the dentin that was being calcified during the immediate effect of the first injection and a secondary hypercalcified stripe in the dentin that was being calcified subsequently. The extent of the secondary stripe varied with the number and unitage of the doses injected and the duration of the experiment.

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* The preparation of a portion of the hormone used in this report was aided by a grant from the Committee on Scientific Research of the American Medical Association to W. R. T. and by a supply of parathormone to F. A. McJ. from Lilly & Company.

Two animals that were given 12 daily injections of 10 units each of parathyroid hormone showed a normal histologic picture.

The absence of reaction in the 3 animals that were given inactivated hormone preparation gives conclusive proof that the effects observed were due to the parathyroid hormone and not to any foreign protein impurities.

It is believed that the experimental pattern in the dentin represents its response to the changes in the calcium and phosphorous metabolism induced by the injections of the parathyroid hormone. The dentin reaction can be explained best by the theory that the parathyroid hormone controls a fraction of the serum calcium. The findings suggest the feasibility of a method of standardization of parathyroid hormone based on the changes shown to occur in the dentin.

7195 P

A Method for the Assay of Commercial Gastric Mucin.

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From the Departments of Experimental Surgery and Biochemistry, Northwestern University Medical School.

Following the introduction by Fogelson[1] of gastric mucin therapy for gastro-duodenal ulcerative disease, a method for the assay of commercial preparations became desirable.

The commercial gastric mucin is prepared by following the customary procedure for commercial pepsin, in which the mucin dough is separated from the pepsin by precipitation at its iso-electric point. It is then purified by repeated washing with 70% alcohol,[2] mixed with required base, and diluted with peptone to decrease the viscosity to a practical state for administration to patients. Although it is the opinion of Hurst[3] that "mucus differs from other proteins in the extreme slowness with which it undergoes peptic digestion . . . ," nitrogen and reduction values to be presented for commercial mucin suggest the possibility of a partial digestion, when compared with values obtained for gastric mucin by Webster and Komarov.[4] This phase of the subject is being investigated.

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[1] Fogelson, S. J., *J. Am. Med. Assn.*, 1931, **96**, 673.

[2] Ivy, A. C., and Jones, K. K., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 218.

[3] Hurst, A. F., *Brit. Med. J.*, 1933, **3784**, 89.

Commercial samples were found to vary greatly as to viscosity, acid combining power, sulphur, nitrogen, and mucoitin content. Methods were therefore sought by which mucin could be separated from the commercial diluents and contaminants before assay. For this purpose we have used an additional peptic digestion followed by precipitation with 70% alcohol. By weighing the precipitated material, comparative values for the mucin content of the samples may be obtained. Our data shows this procedure to give a product with characteristic N_2 content and reduction values. Details of the procedure are as follows: Dissolve 1 gm. commercial mucin and 0.1 gm. pepsin (Wilson's 10,000 units/gram) together in 25 ml. of 0.25% (0.068 N) HCl. This strength acid was found to give a pH approximating 2 with most samples. Digest in the incubator at 37°C. over night, then pipette 10 cc. aliquot samples into weighed 50 cc. centrifuge tubes, and add 28 ml. of 95% alcohol. The contents of the tubes are thoroughly mixed and centrifuged at high speed until the precipitate is well packed (5-10 min.). The supernatant fluid is then decanted, the tubes placed in a vacuum oven at 40-50 mm. pressure, and dried at a temperature not exceeding 50°C. Constant weight is obtained with approximately 6 hours' drying. If it is desirable to decrease the time of drying, the precipitate may be washed with higher concentrations of alcohol and lastly with ether, before being placed in the oven.

TABLE I.
Percentage Precipitation, Nitrogen, and Reduction Values for Crude and Purified Commercial Mucin.

	Crude Commercial Mucin			Purified Product	
Sample	% Ppt. after Purifying Process*	N. %	Reduction % as glucose	N. %	Reduction % as glucose
1	27.1	13.3	14.7	7.7	35.1
2†	69.0	8.0	28.2	7.2	33.8
3	41.4	12.2	18.9	7.2	37.9
4	30.6	12.6	13.4	7.5	35.6
5	58.8	9.3	22.3	7.4	33.8
6	39.2	11.2	16.8	7.2	37.9

*Percentage of crude sample precipitable by 70% alcohol after peptic digestion.

† Sample washed with 70% alcohol by manufacturer and submitted without addition of diluent.

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[4] Webster, D. R., and Komarov, S. A., *J. Biol. Chem.*, 1932, **96**, 133.

Samples of gastric mucin from various commercial sources when treated by the above procedure give closely agreeing values for nitrogen content and reduction after hydrolysis. Representative data are presented in Table I.

The hydrolyzed samples for analysis are prepared by weighing out 0.1 gm. of crude or purified mucin into a 125 ml. Erlenmeyer flask. To this 50 ml. of 2 N sulfuric acid is added, and the sample hydrolyzed in a boiling water bath for 2½ to 3 hrs., using an air condenser.

For estimation of reducing substances, Somogyi's modification[5] of the Shaffer-Hartmann method using the copper reagent No. 2 is employed. Two ml. aliquots of the acid digestion mixture are pipetted into the test tubes, 1 drop of phenolphthalein added, and the sample neutralized with normal KOH or NaOH. Five ml. of the copper reagent are then added and the analysis conducted as for blood sugar. Results are expressed as percentage reduction calculated as glucose.

For the estimation of nitrogen the method of Koch and McMeekin[6] is employed. Usually 1 cc. of the hydrolyzed sample is used for this determination.

7196 P

Gastric Mucin Secretion in Gastro-duodenal Ulcerative Disease.

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It was shown[1] that commercial gastric mucin when subjected to peptic digestion and subsequent precipitation with alcohol at 70% concentration, yielded upon hydrolysis characteristic values for total nitrogen and total reducing substances. This suggested the use of a reduction method for the quantitation of mucin. Obviously before such a method can be applied to gastric juice, it is necessary to remove all other reducing substances, or to use a procedure for obtaining gastric juice not subject to their presence. The former procedure would be capable of wider application and work is in progress with this end in view. Our present interest is in the variations in mucin content of gastric secretion, when juice is obtained from the fasting stomach by means of an alcohol test meal under conditions excluding other reducing substances.

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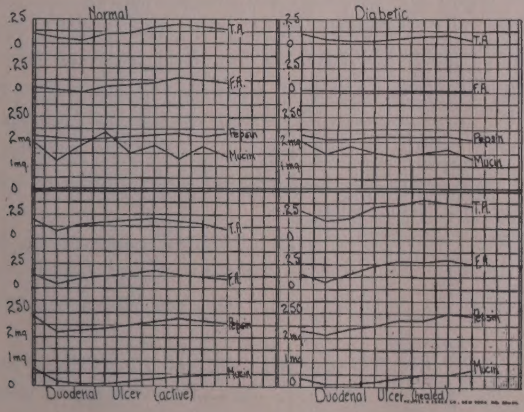
[5] Peters, J. P., and Van Slyke, D. D., *Quantitative Clin. Chemistry*, Baltimore, 1932, **2**, 469.

[6] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

[1] Anderson, R. K., Fogelson, S. J., and Farmer, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 518.

In following the curve of mucin secretion, the stomach is emptied after a night's fast, basal secretory rate established, then 250 cc. of 7% ethyl alcohol is given and samples removed through a Rehfuss tube at 15 minute intervals for a period of 2 hours. Determinations of total and free acidity, and pepsin, are made. The mucin secretion is followed by the determination of total reducing substances according to the following method: The gastric sample (5 cc. if available) is diluted with an equal volume of 4 N H_2SO_4. It is then hydrolyzed in a boiling water bath for 2½ to 3 hours using an air condenser. After cooling, 2 cc. aliquot samples are pipetted into test tubes, the acidity neutralized to phenolphthalein with 1 N KOH or NaOH, and the reduction estimated by the Somogyi modification of the Shaffer-Hartmann method.[2] The results are expressed in milligrams of reducing substances (calculated as glucose) per cubic centimeter of gastric juice.

To illustrate values found, the accompanying graphs are appended.



These graphs illustrate the gastric secretory response in a normal individual, a diabetic with achlorhydria, a patient with an active duodenal ulcer, and one with a healed duodenal ulcer. Gastric contents (after hydrolysis) in the normal and diabetic cases consistently show a reduction of 1 to 2 mg. per cubic centimeter, whereas both ulcer patients are consistently below 1 mg.

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[2] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Baltimore, 1932, **2**, 469.

Although all curves of gastric mucin secretion from patients actively suffering from gastro-duodenal ulcerative disease have not been as classic as the ones reproduced here, they have been consistently of the same pattern and suggest a true quantitative mucin deficiency.

7197 P

Reactions of Hemoglobin and Hematin with Sodium in Liquid Ammonia.

R. G. ROBERTS AND C. O. MILLER. (Introduced by C. J. Farmer.)

From Northwestern University Medical School.

We have shown[1, 2] that certain proteins and peptones are acidic in liquid ammonia and react with sodium with liberation of hydrogen. Proteins and peptones liberate approximately one-half of a molecular weight of hydrogen for each gram atom of nitrogen in the substance when an excess of sodium is used.

Hemoglobin and hematin react profoundly differently toward sodium in liquid ammonia than do the other proteins which we have studied. They liberate hydrogen continuously when excess sodium is present and in quantities disproportionate to the acidic groups present. Their behavior resembles the catalytic action of a rusty iron nail in decomposing the ammonated sodium atom to give sodium ion, amide ion, and hydrogen.

The analytical procedure was essentially the same as that described previously. The substances were dried for 48 hours in an electric oven at 80°C. and then kept in a vacuum desiccator over sulphuric acid.

Hemoglobin is not appreciably soluble in liquid ammonia. It swells, softens, and the particles tend to coalesce. Within a few moments, the supernatant liquid is yellow, but slowly turns to a reddish color after 20 min. After standing for 4 hours, the solu-

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[1] Miller, C. O., and Roberts, R. G., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 535.

[2] Roberts, R. G., and Miller, C. O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 821.

tion is a light green. The change in color may be due to impurities present. There is a persistent increase in rate of boiling of liquid ammonia following the addition of hemoglobin suggesting that ammonation of the molecules is taking place exothermically.

When 0.1 gm. of sodium is added to 0.1 gm. of hemoglobin in liquid ammonia, hydrogen is evolved rapidly during the first 15 min. After that the rate was slowed up considerably, but it proceeded until the blue color of the solution was decolorized. If more sodium was then added, the reaction would start again and continue until the solution was decolorized again. This could be repeated several times. The volume of hydrogen evolved was far in excess of the amount liberated by the same weight of silk, zein, egg albumin, casein, and edestin. The rate is characteristic of a substance containing a carboxyl group, as an amino acid. Hemin contains 2 carboxyl groups, and globin probably contains some free carboxyl groups, although the basic groups in it are in excess. It is possible that our method of drying did not remove all of the water. Water, being acidic in liquid ammonia, reacts rapidly with sodium liberating hydrogen. Hofner reports that 100 gm. of hemoglobin contains 9.25 gm. of water of crystallization.

It seemed likely that the hemin part of the hemoglobin molecule was responsible for the unusual activity rather than the globin fraction because the hemin contains iron, whereas globin is a protein. Two-tenths gm. of hemin prepared from dog hemoglobin was placed in 150 cc. liquid ammonia. It was slightly soluble. The solution turned to dark red. After it had reacted with 0.1 gm. of sodium, it was a light cherry red, and later turned to a dark reddish brown. The first 25 cc. of gas was liberated rather fast at first and then continued slowly for several hours. The sodium was absorbed rapidly. The rate of evolution of the gas resembled that of hemoglobin. Seven hundred cc. of gas was liberated in the first 2 hours with the consumption of 1.4 gm. of sodium. If every hydrogen atom in the sample of hemin had been liberated, this would have accounted for only approximately 100 cc. of gas. The catalytic action of hemoglobin was apparently due only to the hemin fraction. When hemin is added to liquid ammonia in an open Dewar at —33.5°C., the rate of boiling increases. After 2 hours the above solution was greenish in color.

Since iron wire or oxides of iron alone do not have the marked catalytic effect of iron incorporated into the porphyrin molecule as in hemin, hematoporphyrin (from ox blood) was used to see if any catalytic effect was due to porphyrin. One-tenth gm. of hematoporphyrin was added to 150 cc. of liquid ammonia along with 0.1 gm. of metallic sodium. The solution immediately turned blue and remained so for 1½ hours. A few cc. of gas came over during the following 45 minutes, apparently from the pyrrol groups themselves, then all evolution of gas ceased. Hematoporphyrin alone apparently does not have a catalytic effect on the following reaction: $Na + NH_3 = NaNH_2 + \frac{1}{2} H_2$. It is worthy of note that hemoglobin beside its remarkable property of very quickly forming oxyhemoglobin can have such a powerful catalytic action on the system just mentioned and to be able to continue that action in the presence of one of the strongest reducing agents known, the solvated electron.

We think of the marked catalytic activity of the hemoglobin as being due to the presence of the iron in the porphyrin ring, and to that metal's ability to accept electrons from the metallic sodium, thus becoming reduced, and to pass them on to the hydrogen, thereby becoming oxidized again and ready to repeat the cycle.

7198 P

Effects of Partial Adrenalectomy on Experimental Diabetes and on Sensitivity to Insulin.

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In view of evidence (1) that the severity of pancreatic diabetes is greatly diminished by previous hypophysectomy, (2) that hypophysectomy leads to some atrophy of the adrenals and (3) that the adrenals are in some way connected with carbohydrate metabolism, it seemed necessary to reinvestigate in more detail the effects of adrenalectomy on pancreatic diabetes. The literature will not be reviewed here but it is sufficient to say that the reports have been conflicting and inconclusive. Nine dogs have been used in our studies, 5 of which have had the pancreas removed.

In 3 cases the adrenal on one side was removed first and the pancreas removed 10-18 days later. These animals received no insulin and 2 of them are still alive, now 22 days since pancreatectomy. The third animal died from an infection. These animals showed a mild glycosuria but not as severe as is usually found after extirpation of the pancreas. Within a few days the sugar excretion fell to the level found in dogs following hypophysectomy and pancreatectomy. One animal failed to eat part of the time (probably due to a respiratory infection) and under these circumstances no glycosuria was present. In 2 dogs the pancreas was removed first and the animals put on a constant diet with insulin twice daily. Later, one adrenal was removed and in one case the quantity of insulin necessary to maintain the same degree of slight glycosuria was approximately one-half as great as before the adrenalectomy. In the other animal insulin was stopped after the removal of one adrenal and the glycosuria was only mild. The animals are gradually losing weight and becoming weaker, although they apparently feel well and have good appetites. The fasting (overnight) blood sugar of these animals varied from 82 to 290 mg. per 100 cc.

Four other animals from which one adrenal had been removed were injected subcutaneously with 0.5 units of insulin per kg. Three of these animals showed convulsions within 3½ hours. They were soon relieved after the oral administration of glucose. The fourth animal and a normal control which received 1 unit per kg. showed no external symptoms of hypoglycemia.

These results, although not extensive enough to draw conclusions, are similar to what is found after hypophysectomy. The experiments are being continued to see the effects of loss of more adrenal tissue, to test the possibility of the pituitary exerting its influence through the adrenals, and to study other related problems.

7199 P

Heterotopic Formation of Dentine and Enamel by Transplantation of Epithelium and Pulp Tissue.

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From the Department of Surgery, The University of Chicago.

Epithelium surrounding the base of the permanent cuspid teeth, and pulp tissue in 18 young dogs were transplanted to the fascia of the abdominal wall as an autogenous graft, and after 9 to 31 days there was found newly formed enamel, dentine, cementum and bone.

Method. The epithelium was obtained in the following way: Under ether anesthesia a cuneiform osteotomy of the mandible, including the deciduous cuspid tooth was made in dogs of between 3 and 6 weeks of age and this allowed access to the permanent cuspid tooth, which was removed. A circular incision was made equatorially allowing the dental epithelium and pulp to be removed en masse from the calcified tooth shell. The tooth shell itself was always discarded. The soft tissues were gently wiped with moist gauze, were X-rayed, and a section was removed for histological control. The calcium-free material was then transplanted to a pocket between the lateral abdominal muscles, with aseptic precautions, and the incision closed with silk.

It was found easy to separate (a) the sheet of ameloblasts with the subjacent vascular connective tissue parietes, hereafter called the ameloblast layer from (b) the odontoblasts and their underlying pulp cells, in this paper called the odontoblast layer. In some experiments the ameloblast and odontoblast layers were separately transplanted, and in others they were transplanted together.

Results. In all cases calcified tissue was found in the transplant after 9 days. When the ameloblast and odontoblast layers were transplanted together in a nearly normal relationship, enamel and dentine were found between the 2 layers and the ameloblasts were present in a normal cylindrical form, as in the original tissue.

When the ameloblast layer was transplanted alone the cylindrical form of the epithelium was mostly lost, and bundles of enamel rods in large numbers were found scattered through large masses of stratified squamous epithelium resembling gingival epithelium. When the odontoblast layer was transplanted alone, masses of dentine were always found. These in the histological sections cut at 12μ appeared as plaque and circular forms. The circular form was lined inside and out with cells resembling the odontoblasts. In addition there was found in these sections masses of bone, dentine, and of various gradations between dentine and bone, that is atypical dentine and atypical bone.

This effect whereby dentine and enamel are produced after the transplantation of epithelium seems to be similar to the production of bone[1] following transplantation of urinary tract epithelium to the abdominal wall.

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[1] Huggins, C. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 349.

7200 P

Susceptibility of the White Mouse to Tuberculosis.

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The opinion that the mouse is practically immune or at least highly resistant to tuberculous infections seems to be quite generally expressed. Kettle[1] states that the tubercle bacillus is practically non-pathogenic to the mouse in the sense that it does not cause the necrotic or other lesions seen in susceptible animals and does not necessarily kill the animal. Trommsdorff[2] observed that mice were much more susceptible to bovine strains than to human strains when injected intravenously and suggested the possibility of using mice for type differentiation. His work was confirmed by Peters[3] and Binder.[4] Lange[5] used 3 human strains and 2 bovine strains of virulent tubercle bacilli for intravenous injections and observed no marked differences in the susceptibility of mice to the two types.

This report calls attention to the possibility of using white mice for certain types of work in the field of tuberculosis. The relatively low susceptibility of the mouse enhances its value in experiments where chronicity of the disease is desired. The rarity of spontaneous infection and the consistent results from intravenous administration of suitable doses suggest the possibility of a usefulness in virulence tests and possibly for experiments in immunity.

Recently isolated human and bovine strains and a virulent avian strain* were used for intraperitoneal and intravenous injections in full grown white mice. The expectation that mice would be resistant to infection led us to use unnecessarily large doses in the earlier experiments. Two or 3 weeks old cultures were emulsified by shaking with glass beads and the concentrations were made such that the desired dose was contained in 0.5 cc. of saline. In many cases the concentration was reduced by allowing the coarse clumps of bacilli to settle out so that the actual dose given was considerably less than that indicated in the table.

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[1] Kettle, E. H., *J. Path. and Bact.*, 1932, **35**, 395.

[2] Trommsdorff, R., *Arb. a. d. Kais. Gesundheitsamt*, 1909, **32**, 568.

[3] Peters, E., *Zentralbl. f. Bakteriol., Parasitenkrankh. u. Infektionskrankh.*, Abt. I, Orig., 1912, **62**, 1.

[4] Binder, cited by Lange.

[5] Lange, B., *Z. f. Hygiene u. Infektionskrankh.*, 1922, **98**, 229.

* Obtained from Dr. A. J. Vorwald of the University of Chicago. It was originally isolated by Dr. Van Es of the Department of Pathology and Hygiene, University of Nebraska.

In the animals infected with human or bovine strains the tendency for macroscopic lesions to appear in the lungs is striking. Those mice showing advanced pulmonary tuberculosis usually exhibited multiple tuberculous lesions in the liver. Only a few had macroscopic tubercles in the spleen although many showed splenic enlargement. Involvement of the liver and spleen was more frequent with the use of bovine than with human strains.

TABLE I.

	Intraperitoneal		Intravenous		
Human Strains of Tubercle Bacillus					
Dose (moist wt. in mg.)	1.0	0.1-0.25	0.025	0.25	0.025
No. mice studied	40	13	8	6	8
Total No. with macroscopic lesions	90%	69%	25%	100%	87%
No. with macroscopic lesions in lungs	77%	69%	12%	100%	87%
No. with lesions in liver or spleen	22%	15%	12%	17%	12%
Aver. survival period (days)	46	79	120	24	59
Bovine Strain of Tubercle Bacillus					
Dose (moist wt. in mg.)	1.0			0.25	
No. mice studied	16			6	
Total No. with macroscopic lesions	75%			100%	
No. with macroscopic lesions in lungs	69%			83%	
No. with lesions in liver or spleen	44%			66%	
Aver. survival period (days)	45			18	

In the table the results of intraperitoneal and intravenous injections are compared and while the second group is small the data are sufficient to indicate that for a given dose the intravenous inoculations are more effective. The average period of survival is almost precisely the same in mice given 1 mg. of bovine bacilli as in those receiving the same dose of human type. The gross and microscopic appearances of the lesions resulting from the use of the 2 types were indistinguishable. Beginning as discrete, disseminated tubercles, these became confluent and progressed until they occupied one-half to two-thirds of the lung tissue at the death of the animal. Tuberculous pneumonia was not uncommon. Caseating tubercles were unusual in the liver and spleen but caseous nodules in the peritoneum were common in the mice inoculated intraperitoneally.

Seventy-eight mice which received various doses of avian tubercle bacilli through the tail vein, were etherized at different intervals up to 46 weeks. None died as a result of tuberculous infection and the smaller doses resulted in no gross lesions but epithelioid tubercles and lymphocytic infiltration were found in the microscopic sections of liver and spleen in most cases. When large doses (1 mg.) were used, characteristic lesions appeared almost invariably. The liver and spleen were enlarged but the lungs were usually free from gross lesions. Caseation never occurred in the avian infections.

7201 P

State of Calcium in Protein-containing Fluids.*

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We have investigated the nature of the relationship between calcium and protein in blood serum and other protein-containing fluids, using the frog-heart method, previously described,[1] for direct estimation of calcium ion concentrations, and have accumulated evidence concerning the hypothesis[2] that there is a substance, like citrate, and other than protein, which binds calcium in serum and other fluids of the body. When purified electrolyte-free serum proteins (beef) are dissolved in solutions of approximately the electrolyte concentration of the serum, calcium combines with the protein in a manner analogous to that previously described for citrate.[3] The combination represents an equilibrium between protein, total calcium, and calcium ions, and this equilibrium may as a first approximation be described by the mass law equation

$$\frac{[Ca^{++}] \times [Prot^{=}]}{[CaProt]} = K \qquad (1)$$

in which

Ca^{++} = calcium ion concentration—mols. per kg. water.
† $Prot^{=}$ = base combining capacity of protein present at pH of solution, in equivalents × 0.5, less equivalents combined with calcium, per kg. of water
CaProt = calcium combined with protein expressed as mols of Ca per kg water.
K = constant.

In logarithmic form this equation is written as $pCa^{++} + pProt^{=} - pCaProt = pK_{CaProt}$ in which p indicates the negative logarithm.

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* This work has been aided by a grant from the Josiah Macy, Jr., Foundation.

[1] McLean, F. C., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1344.

[2] Greenwald, I., *J. Biol. Chem.*, 1926, **67**, 1.

[3] McLean, F. C., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1136.

† The assumption, implied by the equation, that the base combining equivalents of protein behave as though they were divalent ions is purely empirical, in order to fit the data at hand.

TABLE I.
Calcium Ion Estimations.
Human Material.

Case	Fluid	Total Ca mM/Kg H_2O	Total Protein %	Ca^{++} — mM/Kg H_2O			Diagnosis
				Calc.	Obs.	Diff.	
B	Serum	4.50	7.44	2.04	1.90	—.14	Unexplained Hypercalcemia
K	”	1.59	(6.17)	0.73	0.50	—.23	Uremia and Tetany
	”	1.81	(6.17)	0.83	0.60	—.23	Same—negative Trousseau
	”	1.28	(6.17)	0.59	1.15	+.56*	After recovery
R	”	2.40	6.31	1.12	1.15	+.03	Rickets
W	”	1.95	4.00	1.15	1.25	+.10	Nephrosis
	Pleural Fluid	1.62	1.80	1.25	1.25	—	”
	Ascitic Fluid	1.64	2.13	1.20	1.25	+.05	”
A	Edema Fluid	1.49	0.33	1.43	1.35	—.08	”
G	Serum	1.89	3.10	1.25	1.45	+.20	Amyloid nephrosis *in extremis*
S	”	2.16	5.11	1.12	1.20	+.08	” ”
M	”	2.80	7.73	1.15	1.10	—.05	Normal
	”	2.99	7.56	1.28	1.30	+.02	”

*Probably experimental error. Note: protein analyses not simultaneous.

When normal human serum is diluted with a solution containing approximately the electrolyte concentration of the serum, but without calcium, and when calcium is added to the various dilutions, the same relationship is found. From observations on both serum and purified serum proteins at pH 7.35 (approximately) a value of 2.22 has been derived for pK_{CaProt} when grams of protein are reduced to base combining capacity by use of the factors given by Van Slyke, Hastings, Hiller, and Sendroy.[4] Agreement of the values for pK_{CaProt} obtained from protein-containing fluids and from purified serum proteins indicates that it is unnecessary to postulate any appreciable amount of other calcium-binding substances in human serum.

Table I includes all observations on undiluted human material in which total calcium, total protein, and calcium ions have been determined upon the same specimens. In all cases the factor used for reducing grams of protein to base combining capacity at pH 7.35 is 0.243, which assumes a normal distribution of albumin and globulin. Further work may indicate the desirability of applying separate factors to these proteins.

These observations, while still few in number, include a wide range both of total calcium and total protein. Further work is required to establish the significance of such discrepancies between the calculated and observed calcium ion concentrations as are included in Table I.

Additional evidence that the frog-heart method is specific for ionized calcium, other conditions being equal, and that calcium bound to protein is physiologically inactive with respect to the frog-heart preparation, has been obtained from the observation that when citrate is added to solutions of purified proteins, or to serum, the values found for calcium ions satisfy simultaneously the mass-law equations for calcium citrate and for calcium proteinate.

For human material it is concluded that the protein-containing fluids may, from the standpoint of the state of calcium, be regarded as solutions of calcium proteinate, which dissociates as a weak electrolyte into calcium and protein ions, with an undissociated fraction of calcium proteinate, the degree of dissociation depending upon a mass-law relationship. At any given pH, therefore, the concentration of calcium ions is determined by the total calcium and the proteins present. Other substances binding calcium in human material, if any, must be in relatively low concentrations, unless as a result of unusual conditions.

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[4] Van Slyke, D. D., Hastings, A. B., Hiller, A., and Sendroy, J., Jr., *J. Biol. Chem.*, 1928, **79**, 769.

7202 C

Effect of Heparin on Acute Anaphylactic Shock.

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Kyes and Strauser[1] studied the effect of heparin on acute anaphylactic shock in the pigeon and concluded that heparin as a reagent inhibiting fibrin formation so markedly eliminates anaphylactic shock reactions as to give support to the view that there is a causal relation between fibrin emboli and the symptoms of shock. There are conflicting reports on the effect of heparin on anaphylactic shock in the guinea pig, Williams and Van de Carr[2] reporting that heparin prevents or reduces the shock symptoms while neither Reed and Lamson[3] nor Hyde[4] found any such protection. So far as we are aware, similar experiments have not been performed on the dog. Dogs were sensitized to horse serum. After varying intervals of time, the dogs were anesthetized with ether, the femoral vein exposed for injections and the carotid artery cannulated for recording blood pressure. Heparin in saline was then injected intravenously and after varying time intervals the critical dose of serum was injected. The details of the experiments and the results appear in Table I.

In 39 control experiments in which the amounts of the sensitizing and shocking doses and the time intervals are fairly approximate to those here reported, the distribution of the varying grades of severity of shock were as follows: ++++ 28%, +++ 13%, ++ 44%, + 8%, and 0 3%. In the heparin series, the corresponding ratios are ++++ 6%, +++ 12%, ++ 64%, + 6%, and 0 6%. We do not believe that the difference between the 2 series is significant. Certainly there is not the marked modifying influence of heparin reported by Kyes and Strauser in the case of the pigeon or by Van de Carr and Williams in the case of the guinea pig. Consequently we conclude that these experiments lend no support to the theory that the formation of fibrin emboli condition the symptoms of anaphylactic shock in the dog.

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[1] Kyes, Preston and Strauser, *J. Immun.*, 1926, **12**, 419.

[2] Williams and Van de Carr, PROC. SOC. EXP. BIOL. AND MED., 1927, **27**, 793; Van de Carr and Williams, *J. Immun.*, 1928, **15**, 13.

[3] Reed and Lamson, *J. Immun.*, 1927, **13**, 433.

[4] Hyde, *Am. J. Hygiene*, 1927, **7**, 614.

TABLE I.

Effect of Heparin on Acute Anaphylactic Shock in the Dog.

Exp. No.	Wt. Kilos	Sensitizing Dose		Interval Days	Shock Dose cc.	Heparin in mg. per kilo	Time between Heparin and Shock, min.	Time Onset Shock Seconds	Degree Shock
		Intraven. cc.	Subcu. cc.						
41	17	4.5	—	24	10	10	8	90	++*
68	13.6	13	5	97	13.6	15	15	90	+++
69	19	10	—	15	19	10	4	90	++
70	19	10	—	15	19	10	4	30	++++
71	12.3	10	—	15	12.3	10	6	50	++
72	7.7	10	—	15	8	10	9	35	+++
73	11	10	—	15	11	10	6	?	+
74	19	10	—	15	19	10	5	38	++
75	18	10	—	15	20	10	6	32	+++
76	10	5	5	13	10	10	8	—	O
77	10	5	5	13	10	10	8	32	++
81	17.2	5	5	20	10	10	35	45	++
82	8.2	5	5	26	2.2	10	20	55	++
83	8.5	5	5	27	4	10	15	60	++
84	15	5	5	15	7.5	10	18	30	++
85	12.3	5	5	15	6.2	10	10	35	++

* Method of grading shock.
O = no recognizable shock symptoms.
+ = Insignificant fall in blood pressure. Contraction of urinary bladder or other evidences of shock present.
++ = Moderate to marked fall in blood pressure, with partial or complete recovery in 20 minutes.
+++ = Marked fall in blood pressure. No recovery in 30 minutes.
++++ = Death in less than 30 minutes.

7203 P

Formation of an Epinephrin-like Substance in Autolysing Adrenal Glands.

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Fresh whole adrenal glands (20 gm. portions) were thoroughly crushed in a mortar with sand (3 gm. sand per gm. of gland) and 50 cc. distilled water, until a very finely divided pulpy mass was formed. This pulp was placed in collodion bags and dialyzed repeatedly against 200 cc. portions of distilled water. Dialyzing for 5 minutes results in a bluish dialysate, 5 cc. of which causes a powerful pressor response when injected intravenously in a 10 kg. dog. The dialysates have been removed and fresh portions (200 cc.) of distilled water added daily at varying intervals for 35 days. Intravenous injections in a 10 kg. dog of ½ to 5 cc. of the different dialysates causes a rise in blood pressure comparable to the effect obtained by injecting ½ to 1 cc. of 1/20,000 P. D. and Co. adrenalin. Chemical analysis, according to the iodine thyosulphate titration, indicates that the total yield per gram of fresh gland over 30 days' dialyzing is equivalent to 10 mg. P. D. and Co. adrenalin. Biological assay as determined by the blood pressor effect on anesthetized dogs, shows even a greater physiological effect than the quantitative chemical analysis indicates.

If autolysis of the crushed adrenal glands in the dialyzing bag be held in abeyance by potassium cyanide, the dialysate contains no pressor principle. On the other hand KCN did not affect the pressor activity of untreated dialysates nor that of P. D. and Co. adrenalin. Boiling for 1 minute does not destroy the pressor effect of the dialysate; neither does digestion with pepsin or trypsin. Passing the dialysate through animal charcoal removes the pressor principle, as does adsorption on aluminum hydroxide.

The pressor substance contained in the dialysate is not precipitated by concentrated NH_4OH, although a heavy flocculent precipitate forms. The mother liquor from this precipitate contains a powerful pressor substance.

Conclusion. Autolysis of crushed, whole adrenal glands gives rise to a powerful pressor substance which may be obtained by dialysis. This substance gives all the ordinary chemical tests for adrenalin, but differs from it in that it is not precipitated by concentrated ammonium hydroxide. It does not give a biuret reaction; is not destroyed by boiling; is adsorbed by animal charcoal and aluminum hydroxide and does not lose its physiological effect if exposed to air at room temperature for 30 days. Glacial acetic acid does not destroy its pressor effect. Inhibiting autolysis of the crushed adrenal glands by the addition of potassium cyanide prevents the formation of the pressor substance as determined by chemical and physiological tests.

7204 P

Effect of Pancreatectomy on Glutathione Content of Blood.

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Complete pancreatectomy in the dog causes an increase of over 100% in the non-glucose reducing substance of venous blood. (Table.) This substance is determined quantitatively by the iodine titration method of King *et al.* for blood glutathione as modified by Mancorps and Schmid.[1] At this stage of the research we assume this substance to be glutathione; however, the method employed is not specific but may include such substances as thioneine and cystein. Ascorbic acid also interferes with this titration. The above mentioned interfering substances are probably not present in quantities sufficiently great to cause appreciable error in our interpretations. Further work on this phase is in progress.

TABLE I.
Effect of Removal of Pancreas on Blood Glucose and Glutathione.

	Before removal of pancreas		After removal of pancreas									
			4 days		6 days		10 days		13 days		15 days	
Dog No.	Glucose mg. %	Glutathione mg. %	Glucose mg. %	Glutathione mg. %	Glucose mg. %	Glutathione mg. %	Glucose mg. %	Glutathione mg. %	Glucose mg. %	Glutathione mg. %	Glucose mg. %	Glutathione mg. %
5	83	24.6	247	28.8	229	46.0	280	31.9	272	27.6	200	28.2
6	80	19.6	256	35.0	165	41.1	260	36.6	254	21.7	241	30.0

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[1] Mancorps, C., and Schmid, R., *Hoppe-Seyler's Z. fur Physiol. Chemie*, 1932, **205**, 141.

Section of the splanchnics in the dog causes a decrease of approximately 50% in the blood glutathione, but no effect on the *experimental diabetes* following *pancreatectomy*. Acetone given by mouth in dosages of 6 cc. per kilo of body weight causes this glutathione-like substance to increase approximately 200% above normal and does not return to normal for approximately 3 days. Removal of the pancreas on the blood glutathione has been studied by Dott D. Ferrari.[2] Our results are at variance with his findings. Blood glutathione determinations before pancreatectomy in his dogs are not published.

7205 P

Influence of Bleeding, Diet, Distemper, and Starvation on Serum Phosphatase Activity.

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In the course of a study of serum phosphatase activity in the rabbit, dog and rat, it was found that bleeding, diet, distemper, and starvation exert a marked influence upon the values obtained. Bodansky and Jaffe[1] have pointed out that a relationship exists between the type of diet, the nutritional state of the animal, and the plasma phosphatase activity. For convenience of presentation, data is given under the 4 respective headings. Serum phosphatase activity was determined according to the method of Bodansky.[2]

1. The influence of bleeding upon serum phosphatase activity was determined in adult rabbits. The serum obtained from 25 cc. of blood, drawn from the heart of rabbits, gave average values in a group of 14 animals for calcium of 13.96 mg./100 cc., for inorganic phosphorus 5.04 mg./100 cc., and for phosphatase activity 4.14 mg./100 cc. A second 25 cc. sample of blood drawn 8 to 15 days later showed the influence of the previous bleeding upon serum constituents of otherwise normal rabbits maintained on the same diet. The values for serum calcium averaged 14.13 mg./100 cc., for inorganic phosphorus 3.74 mg./100 cc., and for phosphatase

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[2] Ferrari, Dott R., *Boll de Soc. Med.-Chir.*, Pavia, 1933, **47**, 305.

[1] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 199.

[2] Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 760.

activity 2.25 mg./100 cc. These data show that bleeding caused a marked decrease in serum phosphatase.

2. The influence of a high protein or carbohydrate diet on the serum phosphatase activity was studied. The diet of a number of dogs was alternated from bread and meal mash to one of beef hearts. The influence of these 2 diets on the serum phosphatase and inorganic phosphorus is shown in Table I.

TABLE I.

Influence of Diet on Serum Inorganic Phosphorus and Phosphatase.

Dog No.	Inorganic Phosphorus mg.	Phosphatase Activity mg.	Days on Diet	Diet
9	4.05	14.00	18	bread and meal
	4.92	3.86	13	beef hearts
	3.82	6.81	14	bread and meal
	3.18	12.51	13	” ” ”
	3.85	3.27	14	beef hearts
	4.76	11.22	15	bread and meal
10	7.30	15.25	15	” ” ”
	6.75	4.77	14	beef hearts
	6.75	20.62	14	bread and meal
	6.77	4.61	15	beef hearts
	5.12	6.94	14	bread and meal
	5.46	16.47	15	” ” ”

These results show that a high serum phosphatase activity accompanies feeding a bread and meal diet (high carbohydrate), while a beef heart (high protein) diet induces a low phosphatase activity. (The serum inorganic phosphorus sometimes varied directly and at other times inversely with the phosphatase activity.) Dogs when upon the lean meat diet invariably gained weight.

The influence of diet on the serum phosphatase of rats was also studied in an attempt to repeat the observations of Bodansky and Jaffe,[1] who found that rats maintained on a lean meat diet for 30 days showed a higher plasma phosphatase activity than those fed Sherman's Stock Diet the same length of time. Two groups of 8 rats each were placed on respective diets. Rats 1 to 8, inclusive, were placed on Sherman's Stock Diet No. 13, and rats 9 to 16, inclusive, were placed on the following diet:

2500 gm.	ground beef hearts
25 gm.	calcium carbonate
30 cc.	cod liver oil
200 cc.	tomato juice

All animals received as much food as they would eat. After 30 days on the 2 rations, the rats were killed, and the blood and serum analyzed. On the serum, calcium, inorganic phosphorus, and phosphatase were estimated, while the hematocrit reading, organic and inorganic phosphorus values were determined on whole blood. The weight increase, as well as the analytical values, were practically identical for the 2 groups with the exception of the phosphatase activity, which was 46.40 for the group maintained on Sherman's Stock Diet as compared to 36.80 for those fed lean meat.

3. The influence of distemper on the serum phosphatase activity was observed in 3 dogs upon which normal values had been obtained previously. Table II shows the increase in phosphatase activity which accompanied distemper in these animals.

TABLE II.

Dog No.	Phosphatase Values Normal mg./100 cc.	Phosphatase Values Distemper mg./100 cc.
1	5.78	17.60
2	3.51	7.15
3	5.08	11.39

4. The influence of starvation on the serum phosphatase activity was observed in 3 dogs that had previously been on a diet of bread and meal mash. The serum phosphatase values prior to, and after, 5 days' starvation are given in Table III.

TABLE III.

Dog No.	Phosphatase Values Before Starvation mg./100 cc.	Phosphatase Values After 5 Days' Starvation mg./100 cc.
6	7.04	3.95
10	16.47	12.71
9	11.22	6.30

Bodansky and Jaffe[1] reported a decrease in the plasma phosphatase activity of guinea pigs starved for 3 and 6 days, as well as of rats starved for 96 hours.

The results that we have obtained as to the influence of a lean meat diet on the serum phosphatase activity differ from those reported by Bodansky and Jaffe.[1] When dogs or rats are maintained on a high carbohydrate diet, the serum phosphatase activity is higher than when these animals are fed a diet rich in meat. The explanation of this fact probably exists in the metabolism of these 2 types of food. The decreased phosphatase activity observed in starvation may be due to an increase in endogenous protein metabolism. The serum phosphatase decrease which results from bleeding may have a similar explanation, while the increase observed in distemper may be due to the liberation of this enzyme from disintegrating leucocytes, since Roche[4] has shown these cells to contain this enzyme.

7206 C

Methemoglobin and Methylene Blue as Cyanide Antagonists.

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The action of methylene blue in restoring the respiration of cyanide inhibited tissues (Gerard[1]) has led to the clinical and apparently successful use of the dye as an antidote for cyanide poisoning (Brooks[2]). It has been urged that methylene blue acts in the intact organism not directly by substituting in the cells for the inactivated respiratory enzymes, but indirectly by removing the cyanide and releasing the enzyme. Cyanide would thus be removed by the formation of the stable cyanmethemoglobin, from it and methemoglobin; formed in turn from the blood pigment by methylene blue (Wendel[3]). An antagonism of methemoglobin and cyanide has been demonstrated on rat tissues (Rosenthal and Voegtlin[4]).

It seemed of interest, in this connection, to determine the action of methemoglobin and methylene blue, with cyanide, on isolated invertebrate tissues. Ciliated gill tissue of the quahog *(Venus mercenaria)* was isolated in sea water and its oxygen consumption followed at 22°C. in Warburg manometers. Alkali in the inset, by absorbing CO_2 and HCN, led to a rise in pH, but always less than 0.5.

The normal Q_{O_2} (cmm. O_2 per hour per gm. fresh weight) averaged 375 (26 experiments) during the first hour in sea water, about 10% less for the second. Cyanide led to typical inhibition, though rather high concentration was needed. As per cent of the normal, average (4 series of experiments) values in cyanide were: M/11,000,

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[3] Sherman, H. C., and Munsell, H. E., *J. Am. Chem. Soc.*, 1925, **47**, 1639.

[4] Roche, J., *Bull. soc. chim. biol.*, 1931, **13**, 841.

[1] Gerard, R. W., *Physiol. Rev.*, 1932, **12**, 504.

[2] Brooks, M. M., *Am. J. Physiol.*, 1932, **102**, 145.

[3] Wendel, W. B., *J. Biol. Chem.*, 1933, **100**, Proceedings C.

[4] Rosenthal, S. M., and Voegtlin, C., *Public Health Reports*, 1931, **46**, 521. .

66; M/5500, 58; M/1100, 56; M/550, 55; M/110, 16. Methylene blue in 0.09% (M/350) concentration, increased the respiration in sea water by one-third. (One-tenth this concentration was without effect, even after cyanide.) Respiration inhibited by M/110 cyanide to 16% normal was more than doubled (to 38%) by the dye, though still depressed. This dye concentration did, however, accurately restore to normal the respiration inhibited (to 57%) by M/1100 cyanide (4 experiments). It is to be noted that considerable restoration occurred even when the added dye was of lower molarity than the cyanide, indicating its separate action as a substitute catalyst in the cell.

Methemoglobin, on the contrary, only affected cyanide inhibition when an excess of the pigment was present. Ox erythrocytes in Ringer were treated with amyl nitrite, washed repeatedly and suspended in Ringer or sea water at approximately blood volume. Probably most of the hemoglobin had been oxidized, so that the suspension, containing about 15% methemoglobin was roughly M/500. It was diluted 11 times as used. Such a suspension had no influence on the respiration of gill tissue. (A similar suspension of hemoglobin cells increased respiration some 25%. This could not have been due in large degree to better oxygenation of the solution in the presence of the carrier. Cyanide inhibition was not affected). With sufficient methemoglobin, the cyanide inhibition was completely reversed or, if the pigment was added with the cyanide, prevented. As estimated above, one mol. of the pigment neutralizes 2 to 4 of cyanide. (Some cyanide distills into the alkali cup in time, so that after an hour or two the smaller methemoglobin concentrations are effective.) Results are given in Table I.

TABLE I.

Concentration		O_2 consumption in % of control		Final color
Cyanide M/	Methemoglobin M/	Before adding Methb.	After adding Methb.	
—	5500	100	95*	brown
5500	5500	62	100	reddish-brown
1100	5500	57	71	red-brown
550	11000	55	62	bright red
”	5500	”	60	” ”
”	2200	”	67	red, brown tinge
”	1100	”	100	reddish-brown

* Each value is the average of 4 experiments.

That a simple chemical reaction of methemoglobin with cyanide is involved is further suggested by the facts that, (1) the methemoglobin remains in the erythrocytes so that it could not readily act upon the gill cells, and (2) only when the brownish color of the methemoglobin remains after mixing with cyanide is the inhibition removed; when an excess of the cyanide converts all the pigment to the bright red cyanmethemoglobin, inhibition remains.

Under the conditions of these experiments, therefore, it appears that methylene blue antagonizes cyanide inhibition by substituting for the cyanide poisoned respiratory enzyme; methemoglobin by chemically removing the active cyanide and freeing the poisoned enzyme. In the intact vertebrate, methylene blue may well act indirectly via the formation of methemoglobin.

7207 C

Electrical Stimulation of the Hypothalamus.

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Evidence has been presented that stimulation of the hypothalamus causes pupillary dilatation,[1, 2] increase in blood pressure,[3, 4] cardiac arrhythemia and ventricular extrasystoles,[4] and contraction of the bladder.[5] The suggestion has been made that the parasympathetic and sympathetic divisions of the autonomic system are separately represented in the hypothalamus.[6, 7] We have shown that the mechanism yielding pupillary dilatation is not sharply localized at one point but is widespread throughout the hypothalamus and that the most marked reactions are obtained from the lateral part in the region occupied by the medial forebrain bundle.[2]

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[1] Karplus, J. P., and Kreidl, A., *Pflüger's Arch. f. d. ges. Physiol.*, 1928, **219**, 613.

[2] Ranson, S. W., and Magoun, H. W., *Arch. Neurol. and Psychiat.*, 1933, **29**, 1179.

[3] Karplus, J. P., and Kreidl, A., *Pflüger's Arch. f. d. ges. Physiol.*, 1927, **215**, 667.

[4] Beattie, J., Brow, G. R., and Long, C. N. H., The Vegetative Nervous System, Vol. XI. Association for Research in Nervous and Mental Disease. Williams and Wilkins, Baltimore, Md. 1930.

[5] Karplus, J. P., and Kreidl, A., *Pflüger's Arch. f. d. ges. Physiol.*, 1909, **129**, 138.

[6] Cushing, H., Papers Relating to the Pituitary Body, Hypothalamus and Parasympathetic System. Thomas, Baltimore, Md. 1932.

[7] Beattie, J., *Canadian Med. Assn. J.*, 1932, **26**, 400.

Directing our attention to this problem again, we have explored the hypothalamus millimeter by millimeter in 44 cats using a faradic current delivered through a bipolar needle electrode accurately placed with the Horsley-Clarke stereotaxic instrument. The technique was the same as that employed in our previous experiments[2] except that changes in respiration, blood pressure, and bladder tonus were recorded on a kymograph. The results of this new series of experiments confirm our previous observation on the pupil and respiration. Pupillary dilatation and increases in rate and depth of respiration were elicited along with a rise in blood pressure and contraction of the bladder as a result of the stimulation. Better reactions were obtained from the lateral part of the hypothalamus containing the medial forebrain bundle than from the medial part forming the wall of the third ventricle.

Stimulation farther rostrally in the region of the anterior commissure and adjacent part of the septum also yielded contraction of the bladder. It would appear that the cell masses concerned with this reaction are located here, that from these a descending pathway runs caudalward and that bladder reactions elicited from the hypothalamus are due to a stimulation of this descending path. No other parasympathetic responses were elicited by us from the hypothalamus. But often a drop in blood pressure, sometimes associated with a decrease in the heart rate, was obtained from the region of the anterior commissure and adjacent portion of the septum. Stimulation of this same region also caused a decrease in the rate and depth of respiration.

There can be no doubt that the hypothalamus is concerned with the activity of the sympathetic system but we have not been able to show that it has any parasympathetic functions. Perhaps these functions may have their forebrain center farther rostrally in close relation to the anterior commissure.

7208 P

Experimental Peritonitis Produced by Injection of Bacteria.

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Studies of experimental peritonitis have been almost insuperably handicapped by the fact that the injection of bacteria alone has failed to reproduce the disease. This was noted by Wegner.[1] Confirmation by many others has occurred (Pawlowsky,[2] Steinberg,[3] David.[4]) It is a commonplace today that injection of even overwhelming amounts of bacteria fails to produce a peritonitis. Although the animal may be killed by the overwhelming sepsis, the peritoneum does not exhibit any of the usual signs of peritonitis and may even be sterile by the time the animal has succumbed from the absorption of the organism. Rewbridge[5] found that the lethal dose of Welch bacilli injected into the peritoneal cavity was 18 cc. of 24-hour broth culture, but that bacteria in smaller numbers than this had no effect whatsoever.

The standard methods of production of experimental peritonitis are (1) The injection of bacteria plus some foreign body, as agar culture media, feces, etc. (2) Production of internal fistulas such as opening the gastrointestinal tract. (3) Leaving large amounts of necrotic tissue in an infected abdomen such as is produced by the ligation and subsequent gangrene of an appendix. All of these conditions do not as a general principle exactly reproduce the onset of peritonitis in the human clinically.

The experiments tabulated in Table I are important because they demonstrate the fact that it is possible to produce a peritonitis by bacteria alone. Only small amounts of bacteria were used in most cases and the development of a typical peritonitis occurred in about half the cases.

The only difference between these and previous unsuccessful attempts appeared to be that the dogs used were in very poor condition. They were those half-starved animals which arrive in every experimental laboratory and are usually cared for and properly nourished

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[1] Wegner, G., *Arch. klin. Chir.*, 1876, **20**, 51.

[2] Pawlowsky, A. D., *Centr. Chir.*, 1887, **14**, 881.

[3] Steinberg, G., and Ecker, E. E., *J. Exp. Med.*, 1926, **43**, 443.

[4] David, V. C., *Tr. Am. S. A.*, 1928, **46**, 362.

[5] Rewbridge, A. G., and Hrdina, L. S., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 528.

TABLE I.

Material injected	Amount cc.	Death due primarily to generalized peritonitis	Death from other causes	Survival
24 hr. brain culture of *B. welchii* Subperitoneally	1	0	0	1
Same	2	1	1	1
Same	5	0	1	2
Same	4	3	2	2
24 hr. broth culture of *B. coli* Subperitoneally	4	6	3	6
18 hr. broth culture of *B. coli* and *S. hemolyticus* Subperitoneally	5	3	2	1
18 hr. broth culture of *S. hemolyticus* Subperitoneally	5	0	1	4
18 hr. broth culture of *B. coli* Intraperitoneally	5	3	1	0
24 hr. broth culture of *B. coli* Intraperitoneally	4	4	0	14
Same	5	3	1	2
24 hr. broth culture of *S. hemolyticus* Intraperitoneally	5	0	1	2
Same	4	0	0	1
24 hr. broth culture *B. coli* and *S. hemolyticus* Intraperitoneally	5	1	0	3
Totals		24	13	39

for a few days before being used. One may draw the conclusion from these experiments that bacteria alone will cause peritonitis in a high percentage of cases providing that the animal's resistance is lowered by suitable means.

7209 P

Studies on Acholic Cachexia. I.* Use of the Dragstedt Cannula in Biliary Studies.

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Collection of bile from experimental animals has always presented almost insuperable difficulties. Simple intubation of the ducts or the gall bladder has resulted in ascending infection, which has seriously interfered with the results obtained, as the bile then originated in livers more or less damaged by hepatitis. In such preparations leakage and consequent loss of part of the bile nearly always occurs if the experiment is long continued. The Rous[1] type of fistula, in which a long tube is coiled within the abdomen thus putting considerable distance between the bile duct and the skin, has been used extensively in this laboratory with indifferent results. For a few weeks it was often successful, but a very high percentage of cases failed in later months which are the crucial point in these studies.

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* Work done under a grant from the Jessie Horton Koessler Fellowship of the Institute of Medicine, Chicago.

Dragstedt[2] recently reported a new type of fistula which he had used extensively for intestinal studies and for short periods in the gall bladder. It consisted in a gold-plated metal cannula, with a metal disc at the inner end. This was sutured into the intestine. The distal end was then capped with a special small spear point which was pushed through the abdominal wall at a little distance from the laparotomy wound. The intervening space (2-4 inches) was then wrapped in omentum. A screw cap at the distal end enabled one to attach a container.

This principle applied to the gall bladder has functioned with remarkable perfection. The cannula must be considerably longer than that used for intestinal work, as the liver moves on respiration. The total length of our cannulas was 5½ inches. The disc at the inner end was made smaller (¾ inch) and somewhat cup-shaped. After the common duct has been ligated, the cannula is simply implanted into the gallbladder through as small a hole as possible and tied in place. Inversion is not necessary, and not advisable.

This preparation has been used on 7 dogs, 4 of which lived between 4½ and 5 months. Approximately 1,000 daily specimens of bile were obtained and studied, without the loss, even in part, of a single specimen. On 2 or 3 occasions the tube would be blocked, as evidenced by a slight jaundice and absence of the expected amount of bile. A few drops of bile would then appear about the tube. Such blockage is easily overcome by probing the tube. The skin wound invariably remained clean without suppuration. The bile remained perfectly clear and contained no increase in cells. Postmortem examination showed an absence of inflammatory changes in the liver and bile ducts. A football bladder was used to collect the bile. To its nozzle was attached a metal tip which screwed into the cannula. This was sterilized with alcohol when the bag was changed. The latter was carried in a canvas bag slung over the dog's back. The total absence of bile on the skin appeared to do away with the dog's temptation to bite or lick the fistula, and only one dog needed a collar to prevent his biting the bag.

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[1] Rous, P., and McMaster, P. D., A method for permanent sterile drainage of intraabdominal ducts as applied to common duct.

[2] Dragstedt, L. R., *et al.*, *Surg. Gyn. Obst.*, 1933, **66**, 799.

The achievement of such success is especially remarkable in view of the fact that neither of the authors had ever tried the method before and had had no experience whatever with these cannulas. The fact that one dog had 7 pups without interrupting the experiment is significant.

We have no hesitancy in stating that the Dragstedt cannula offers a practically fool-proof method of obtaining nearly 100% of clean specimens from bile fistula dogs, a record that we have never before been able even to approximate.

7210 P

Studies on Acholic Cachexia. II.* Is Anemia a Factor?

ARTHUR D. BISSELL. (Introduced by Edmund Andrews.)
From the Department of Surgery, The University of Chicago.

The question as to whether the well known anemia accompanying acholic cachexia is a primary factor in the disease or only a manifestation of a late or terminal cachexia in general is an important one. On this feature alone are based many of the current theories as to the origin of cachexia cholipriva. One assumption often made is that prolonged loss of bile will deprive the body of the opportunity of resorption of blood-forming pigments from the gastrointestinal tract. The statement may easily be disproved by a comparative study of the rate of fall of the hemoglobin and the cells in acholic anemia. Such an assumption would surely predicate a more rapid fall of the hemoglobin than in the red count. In previously reported series as well as in our tables it can be seen at once that this is not the case.

The other theory is based on the fact that the anemia is a manifestation of lack of fat soluble vitamin, due to inability to absorb such material in the absence of bile salts from the intestinal tract. The evidence for this is admirably summed up by Takasu.[1]

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* Work done under a grant from the Jessie Horton Koessler Fellowship of the Institute of Medicine, Chicago.

[1] Takasu, Misao, *Deutsche Zeitschr. f. Chir.*, 1930, **224**, 240.

Studies of this character have frequently been complicated by the fact that such a surprisingly high percentage of bile fistula dogs develop ulcers of the stomach or duodenum, which are liable to massive hemorrhage. Estimates of this percentage have been given as high as 90% by some observers, although in our own experience it has been very much less. In the experiments here reported this factor was ruled out by post mortem examinations as well as daily search for blood in the stools. The figures reported are from 4 dogs with biliary fistulas who lived from 4½ to 5½ months.

The average loss in body weight during this period was 33%.

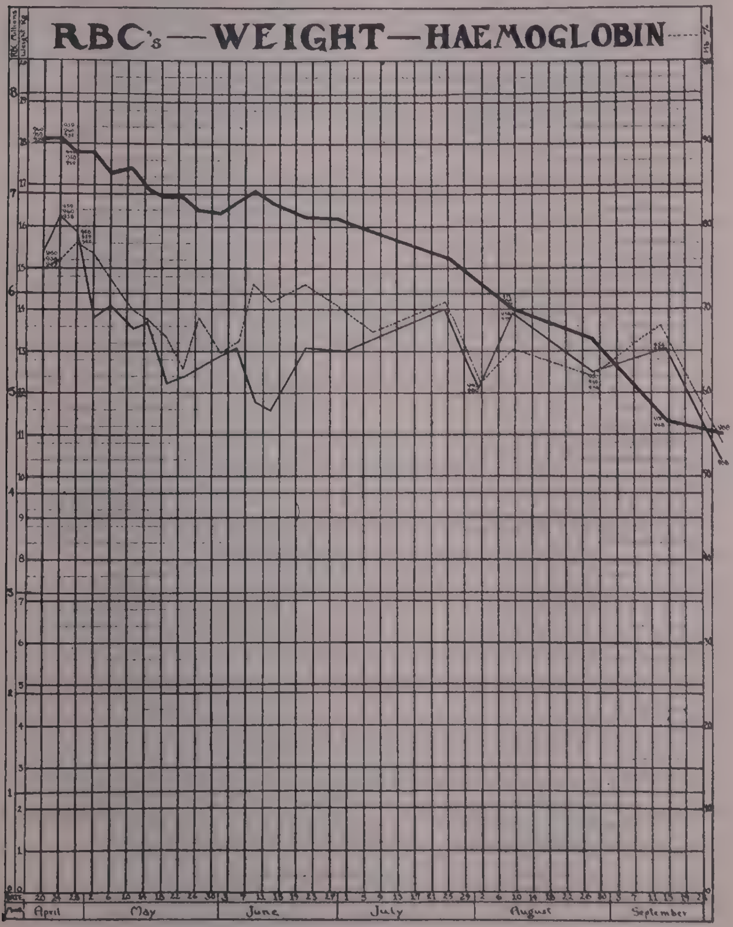

FIG. 1.

The average loss of hemoglobin was 17.4% and of red cells, 17.8%. Four of the dogs died with hemoglobin and red count within the lower limits of normal and the lowest red count was 4,200,000. These findings are certainly not indicative of the fact that anemia is at all a primary factor in the condition. Furthermore, one dog died with a red count higher than before the operation, after having lost 41% of body weight in 5 months, his appearance being in every way typical of acholic cachexia.

Red cell fragility was tested every 4 days in all these animals and in no case was there any deviation from the normal.

From the accompanying chart it is quite clear that the weight loss ran a curve definitely ahead of the anemia, and that while the cachexia and weight loss were profound, the blood findings not only were of a far lesser degree but also did not in the least parallel the progress of the cachexia.

7211 P

Studies on Acholic Cachexia. III.* The Acid-base Equilibrium.

EDMUND ANDREWS AND ARTHUR D. BISSELL.

From the Department of Surgery, The University of Chicago.

For many years it has been assumed that since the bile was alkaline, loss of bile would consequently produce an acidosis. The importance of this factor in acholic cachexia has been a disputed point.

Okada[1] studied fistula bile from dogs and found a pH averaging slightly over 8.00, Düttmann's[2] investigations showed it a trifle lower. In the prolonged studies of Drury, McMaster and Rous[3] duct bile averaged pH 8.20. Neilson and Meyer[4] found figures in the same range, but called attention to the fact that on standing, or heating, duct bile would increase its alkalinity, the pH rising to 8.40-8.60. This was presumably due to driving off of CO_2. Drury[3] emphasized the point that the bile is a well-buffered solution, and that addition of considerable amounts of acid were necessary in

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* Work done under a grant from the Jessie Horton Koessler Fellowship of the Institute of Medicine, Chicago.

[1] Okada, S., *J. Physiol.*, 1915, 1, 114.

[2] Düttmann, E., *Bruns. Beitr. z. klin. Chir.*, 1927, **139**, 720.

[3] Drury, D. R., McMaster, P. D., and Rous, P., *J. Exp. Med.*, 1924, **39**, 403.

[4] Neilson, N. M., and Meyer, K. F., *J. Inf. Dis.*, 1921, 28.

TABLE I.

Days Post-Operative	Cholecystostomy 640		Cholecystostomy 639		Cholecystnephrostomy 745		Cholecystnephrostomy 678		Dragstedt Cannula 824		Cholecystnephrostomy 680		Cholecystnephrostomy 699	
	CO_2	Cl	CO_2	Cl	CO_2	Cl	CO_2	Cl	CO_2	Cl	CO_2	Cl	CO_2	Cl
Pre-op.	%		%		%		%		%		%		%	
Normal	42	294	40	300	36	294	32	283	36		41	305	46	294
4	42	296	42	294	29	299	49	306	33		48	274	39	277
8	40	297	40	272	26	299	34	294	35		27	311	43	250
12	42	307	38	327	23	305	42	289	29		29			
16	37	299	37	283	29	299	32	310	25		28	283		
20	33	—	31	349	32	310	27	305	36		33	316		
24	35	305	35	305	25		32	316						
28	27	305	30	371	30		30	316						
32	33	316	40	327	29		39	332						
36	46	310	46	305	21		33	321						
40	31	348	35	283	25		35	305						
44	31	310	34	327	41									
48	31	327	33	337										
52	36	337	42	300										
56	39	305	24	294										
60	42	310	34	310										
64	40		32											
68	35		42											
72	32		36											
76	36													
80	30													

order to change the reaction. In this connection it is well to remember that considerable fixed acid is lost with the bile, as Wangensteen[5] found that a human with a biliary fistula secreted about 0.4 gm. NaCl daily.

In many previous studies too numerous to mention here, the presence of a mild degree of acidosis has been quite usual, while a few investigators have found it to be of such a high grade as to regard it as a primary factor in the accompanying cachexia. The material here reported consists of CO_2 and blood chloride estimations on 8 animals, part with biliary fistulas and part with cholecystnephrostomies. From the accompanying table it may be seen that there are no significant changes in the acid-base equilibrium. The average CO_2 before operation was 39.5, exactly that taken at the conclusion of the experiments (39.5). The average preoperative blood chloride was 290 mg. per 100 cc. and at the conclusion of the experiments was 293 mg. per 100 cc.

These animals were all on mixed diets and the onset of the cachexia was prompt and typical. It seems, therefore, that one is justified in concluding that if the typical picture may be reproduced without changes in the reaction of the animal that this factor is not fundamental and does not play an etiological rôle in cachexia cholepriva.

7212 P

A Study of the Dog's Stomach and Liver for Substances Effective in Pernicious Anemia.

OSCAR RICHTER, A. C. IVY AND A. F. MEYER.

From the Department of Physiology and Pharmacology, Northwestern University Medical School and Cook County Hospital.

Since gastrectomized dogs do not develop pernicious anemia,[1] it is pertinent to determine whether substances effective in pernicious anemia are present in the liver and stomach of dogs.

We have prepared dog's "desiccated whole stomach" according to the standard method and have given the preparation orally to patients with pernicious anemia. We have made extracts of the dog's liver and have injected them subcutaneously. We have also fed dog's liver to pernicious anemia patients. These materials were prepared from recently sacrificed dogs.

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[5] Wangensteen, O. H., Complete Biliary Fistula, *J. Am. Med. Assn.*, 1929, **93**, 1199.

[1] Ivy, Morgan and Farrell, *Surg. Gynec. and Obst.*, 1931, **53**, 611.

Dog's desiccated whole stomach. In a previous report,[2] the desiccated whole stomach of dogs was fed to 2 pernicious anemia patients in doses of 50 gm. (dry powder) per day for from 8 to 12 days without observing a response. Later both of these patients responded typically, one to oral equine liver and the other to liver extract subcutaneously. We have extended these experiments to include 5 additional pernicious anemia patients, making a total of 7 patients. A slight increase in both red cell count and reticulocytes occurred in one patient. All patients, except one, when placed on equine liver extract responded typically.

Isaacs and Sturgis[3] obtained remissions from feeding from 30 to 60 gm. of desiccated hog's whole stomach. Our results indicate that if the active principle is present in dog's whole stomach, it is present only in smaller amounts than found in the hog's stomach. Larger doses of dog's desiccated stomach were not used because of the difficulty in getting patients to take larger doses voluntarily over a period of adequate length to be significant.

Dog's liver. Six pernicious anemia patients received extracts of dog's liver prepared according to the standard method. The extract of from 45 to 155 gm. of liver was administered subcutaneously during a 10-day period. Three of the patients, one receiving the extract of 45 gm., one 90 gm., the other 140 gm. of liver, showed some evidence of a response, which, however, is questionable. (Table I.)

TABLE I.

Patient	Red Cells Before	Red Cells After	Hemoglobin Before	Hemoglobin After	Reticulocytes Before	Reticulocytes After
R. Bu.	1.26	1.37	34	38	0	0.4
B. Ko.	1.52	1.58	32	34	0.2	2.4
S. Ga.	1.33	1.98	39	49	0	2.0

At least they did not show a progressive decline in the blood picture as did the other patients. (It should be remarked that all patients included in this report had red cell counts varying from 1.7 to 0.7 millions.) We interpret these results as indicating that if the dog's liver contains extractable substances (*i. e.*, extractable by the usual method) effective in pernicious anemia, they must be present in a concentration of less than ¼ of the concentration found in cattle, equine and hog liver.

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[2] Ivy, Richter and Kim, *Am. J. Physiol.*, 1932, **101**, 59.

[3] Sturgis and Isaacs, *J. Am. Med. Assn.*, 1929, **93**, 747.

That the principle effective in pernicious anemia is present in dog's liver is shown by the results to follow. Because it is possible that the effective substance is present in dog's liver but is difficult to extract by the usual method, we fed from 100 to 250 gm. of dog's liver daily to 5 pernicious anemia patients (10 days).

TABLE II

Patient	Red Cells Before	Red Cells After	Hemoglobin Before	Hemoglobin After	Reticulocytes Before	Reticulocytes After	Liver gm. daily
G. Pe.	0.70	0.80	22	22	0	4.2	100
F. St.	1.97	3.35	60	82	0	5.8	200
J. N.	0.67	1.74	18	42	0	18.0	250
M. Re.	1.4	1.92	33	47	0	12.0	250
E. Sc.	1.18	0.80	26	23	0	0.4	200

Three of the 5 (Table II) patients responded without question. One of the patients (G. Pe.) receiving 100 gm. daily failed to respond definitely, and one receiving 200 gm. showed no response. One of these patients (E. Sc.) responded typically to subcutaneous equine liver; the other (G. Pe.) unusually promptly. From these results, it is evident that the material effective in pernicious anemia is present in dog's liver, but very probably not in the concentration (available) found in hog, equine or cattle liver.

Summary. (1) desiccated whole dog's stomach does not contain the material effective in pernicious anemia to the extent that desiccated whole hog's stomach does, and (2) the material effective in pernicious anemia is present in dog's liver, but in less concentration than in cattle, equine and hog liver.

Note: Since our manuscript was submitted, an article by Strauss and Castle (This Journal, p. 360) has appeared which reports that "the content of potent material in canine liver extracts appears to be only about one-fifth that of hog liver."

7213

Lipids of the Thoracic Duct Lymph in Fasting.

BERNARD MORTIMER AND ARTHUR O. TISCHER. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology and the Department of Biochemistry, Northwestern University Medical School, Chicago.

Rony, Mortimer and Ivy[1] reported that the thoracic duct lymph of dogs that had fasted 2 to 14 days contains 250 to 1300 mg. of total fatty acids per 100 cc. lymph with an average value for 60 dogs of 630 mg. %. The total cholesterol content of these lymphs varied from 41 to 114 mg. %.

This work was undertaken to obtain further characteristics of the lipids of thoracic duct lymph in fasting. The lipid phosphorus content, the iodine number and the saponification value of the lymph lipids were examined.

The dogs fasted 4 to 7 days; the thoracic duct was cannulated under intravenous pentobarbital anesthesia and from 20 to 50 cc. of lymph were collected. This lymph was analyzed for lipid phosphorus with the Kuttner-Lichtenstein method,[2] for iodine number with the Hanus method, and for saponification value. Table I gives the results thus obtained on 12 dogs.

TABLE I.
Lipids of Thoracic Duct Lymph in Fasting.

	Total Fatty Acids mg./100 cc.	Total Cholesterol mg./100 cc.	Lipid P mg./100 cc.	"Lecithin" mg./100 cc.	Iodine No.	Saponification Value	% Total Lipids not Saponifiable
Range	250 to 1300	41 to 114	3.86 to 6.27	96.5 to 156	73.7 to 89.25	129.6 to 137.1	30.5 to 33.8
Average	630	71	5.17	129.2	80.26	132.8	32.6

The values given in Table I for total fatty acids and total cholesterol are quoted from Rony, Mortimer and Ivy's paper. The values for "lecithin" are obtained by multiplying the lipid-P by 25. The saponification value represents the mg. KOH required to neutralize 1 gm. of the solids in the ether extract of lymph. The calculation of the unsaponifiable portion of the lymph fat was made on the basis of the assumption that all lipids present are C18 fatty acids.

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[1] Rony, H. R., Mortimer, B., and Ivy, A. C., *J. Biol. Chem.*, 1932, **96**, 737; 1933, **102**, 161.

[2] Kuttner, Th., and Lichtenstein, L., *J. Biol. Chem.*, 1932, **95**, 661.

It is known that, in fasting, lipids are excreted by the intestinal mucosa into the lumen of the bowel (Bloor and Sperry, etc.). Rony, Mortimer and Ivy found that in fasting the thoracic duct lymph fat consists chiefly of this fat, the major portion of which is reabsorbed by the intestinal mucosa and passed into the lacteals and thoracic duct. In view of this it is of interest to compare our data listed above (Table I) with those obtained by Sperry on fecal lipids.[3]

a. Iodine number. Sperry found that the iodine number of the fecal fat of fasting dogs varied between 78.6 and 103 with an average value of 90.4. This is very similar but slightly higher than our figures on lymph fat, the value for lymph being 80.2.

b. Unsaponifiable portion. The fecal fat of dogs kept on a lipid-free diet contains 35 to 40% unsaponifiable lipids, according to Sperry. This compares favorably with our figures on thoracic duct lymph fat in fasting, namely, from 30.5 to 33.8% unsaponifiable substance.

No figures are available for the lipid P content of the fecal fat in fasting.

7214 P

Gastrectomy and Subsequent Hematologic Studies in the Hog.

G. L. MAISON AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Blood studies in gastrectomized animals have been done in the dog[1] and in the rat.[2] The presence of substances effective in pernicious anemia in hog's liver and whole desiccated stomach made it seem worth while to study the effect of gastrectomy in the hog on hematopoiesis. We desire to report our preliminary observations during the past 7 months.

Five Poland China pigs 12½ weeks of age were selected. On the 24th of June, 1933, four of the pigs were gastrectomized by us in the Physiological Laboratory of the University of Wisconsin Medical School at Madison,* one being reserved as control. The distal end of the esophagus was sutured to the side of the first loop of jejunum after closing the proximal end of the duodenum. The operation is more easily performed in the hog than in the dog.

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[3] Sperry, W. M., *J. Biol. Chem.*, 1924, **60**, 261; 1926, **68**, 357.

[1] Ivy, Morgan and Farrell, *Surg. Gynec. and Obst.*, 1931, **53**, 2.

[2] Jung, Maison and Highstone, *Proc. Inst. Med.*, Chicago, 1933, **9**, 389.

One animal died on the day following operation due to peritonitis consequent to spillage of material from a stomach distended with food, the stomach of this hog not having emptied after 12 hours of starvation. The remaining animals were transferred from Madison to our laboratories on their 48th postoperative day. They have been kept in open air cages since their second postoperative week. A second animal died of stricture of the lower esophagus 71 days after operation. This stricture occurred at the site at which the clamp had been applied. The remaining 2 pigs and the control are living and well on their 210th postoperative day.

The results of gastrectomy and of therapy with ferric ammonium citrate on the microcytic hypochromic anemia, which developed to a point requiring treatment in their fourth postoperative month, are summarized in the accompanying table. The diet has consisted of a mixed meal (18% P; 3.5% fat; 45% C; and 7% fiber) made into a thick mush and brought to the boiling point in a steam cooker. This diet contains essential salts, including 0.04% iron oxide, 0.0005% copper sulphate, and 0.0005% potassium iodide. Cod

TABLE I.

	Body Weight Normal	Body Weight Gastrectomized	
Preoperative (6-14-33)	26 lb.	32 lb.	40 lb.
Postoperative (1-7-34)	160 "	68 "	116 "

Mean Hematologic Findings.

	Millions RBC	Hb. Gm. Sahli Method	Hematocrit %	Mean Dia. of RBC	Thousands WBC
In 9 normal pigs	7.26	14.8	44.2	5.51 micra	24.0
Values 120 days after Gastrectomy.					
In 2 pigs before treatment	8.04	11.2	33.6		25.6
Feeding of Iron Started 120th Day after Gastrectomy.					
In 2 gastrectomized animals after 2.4 gm. Fe_3NH_4 citrate per day for 33 days in food	8.16	10.2	30.5	4.58 micra	21.2
Subcutaneous Injections of Iron Started on 154th Day after Gastrectomy.					
In 2 gastrectomized animals after 0.09 gm. Fe_3NH_4 citrate subcutaneously daily for 40 days	8.82	13.1	39.4	5.12 micra	

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* The authors wish to express their obligation to Drs. Meek and Eyster for their generous loan of laboratory facilities and to Mr. John Fargo of the Department of Animal Husbandry of the College of Agriculture of the University of Wisconsin for his kindness in housing the animals as well as his many helpful suggestions relative to the care of hogs.

liver oil was added to this diet. Other pigs are now under way and a more detailed report will be forthcoming when more gastrectomized pigs have been observed for longer intervals.

Summary. In 2 gastrectomized pigs, we have observed the occurrence of a microcytic, hypochromic anemia, which appeared about 3 months postoperatively and which did not respond to 2.4 gm. of iron salt given daily and orally, but did respond to 0.09 gm. of iron given daily and subcutaneously. The growth of the 2 gastrectomized pigs has been retarded.

New York Meeting.

New York Academy of Medicine, February 21, 1934.

7215 P

A Type-Specific Substance from the Meningococcus.

HENRY W. SCHERP AND GEOFFREY RAKE.

From the Laboratories of The Rockefeller Institute for Medical Research.

In an earlier paper,[1] there was described the separation, from meningococcus autolysates, of fractions exhibiting marked type-specificity toward precipitin-containing sera. It is now possible to report the preparation of the Type I specific substance (SSS) in a much higher state of purification.

Twenty liter lots of 3 week cultures of recently isolated strains of Type I meningococcus in hormone broth are acidified with acetic acid to pH 4.0. The SSS has a marked affinity for the acid-precipitable protein, the so-called "P" fraction, and about one-half of the amount present is carried down with the latter under the above conditions. If the broth is diluted 10-fold with tap water before acidification, almost all the SSS is adsorbed, but there is a loss in selectivity. This point is undergoing further investigation.

The "P" fraction is washed with distilled water, suspended in 1 liter of the same solvent and dissolved at pH 7.0 with the aid of NaOH. The bulk of the protein is then precipitated with acetic acid at pH 4.8 and centrifuged off. The precipitate is washed with distilled water and again submitted to the process of solution at pH 7.0 and precipitation at pH 4.8. The combined supernatant fluids, which contain almost all of the SSS, are concentrated *in vacuo* to the smallest practicable volume and precipitated with 5 volumes of alcohol. After standing overnight, the precipitate is centrifuged off and dried *in vacuo* with concentrated H_2SO_4. It is then dissolved in 50 to 100 cc. of distilled water with the aid of NaOH, and freed of the remainder of the protein by 0.7 saturation with $(NH_4)_2SO_4$. The latter is removed by dialysis, 10% of sodium acetate is added and the SSS is precipitated as a yellowish gum by 4 volumes of ethyl alcohol. Further purification is achieved by repeated precipitation by alcohol in the presence of sodium acetate and through precipitation with basic lead acetate or uranyl nitrate. Finally, the SSS is precipitated as a white powder by 10 volumes of absolute alcohol, from aqueous solution containing a small amount of sodium acetate. In favorable cases the yield from 20 liters of broth is 350 mg.

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[1] Rake, G., and Scherp, H. W., *J. Exp. Med.*, 1933, **58**, 341.

At a dilution of 1:2,000,000, preparations such as described give the precipitin reaction with homologous rabbit serum; at 1:8,000,000, they precipitate homologous horse serum. At a dilution of 1:100, slight precipitation occurs with Type II rabbit serum, but not at 1:1,000. Doses of 1 to 3 mg. administered to rabbits intravenously or intradermally have no apparent toxic action.

In 2% solutions the biuret and trichloracetic acid tests are negative. The Molisch test has been positive at 1:10,000 but not at 1:100,000. The substance does not reduce Fehling's solution, but after acid hydrolysis it gives 45% of reducing sugars calculated as glucose. It gives no orcin test for pentoses, and no color with iodine. It is unaffected by tryptic digestion and by short exposure to heat at neutrality, but is very sensitive to acids, the serological activity being destroyed by heating for one-half hour at 100° C., at pH 4.0. Standing for one hour at room temperature in N HCl has the same effect. At the same time, about 7% of reducing sugar appears calculated as glucose. The specific rotation, $[\alpha]_D^{25^\circ C.}$, is $+45^\circ$. The SSS does not dialyze appreciably through cellophane or parchment but does pass through collodion membranes prepared in the customary manner. It is precipitated by uranyl nitrate, basic lead acetate and safranin but not by copper sulfate, mercuric chloride, strong barium hydroxide solution, saturated ammonium sulfate or saturated calcium chloride.

The substance contains 4.4% N and 6.5% P. The latter is firmly bound since no precipitate is formed with the molybdate reagent until the SSS is subjected to vigorous hydrolysis. On ignition the SSS leaves 29 to 30% ash which present evidence shows to be an alkaline earth phosphate, probably that of calcium. The ash content is unchanged by prolonged dialysis.

Further studies on the chemical and immunological properties of this substance are in progress.

7216 P

Anaphylactogenic Properties of Purified Milk Proteins, Raw, Evaporated, Superheated, Dried and Acidified Milk.*

BRET RATNER AND H. L. GRUEHL.

From the Departments of Pediatrics and Immunology, University and Bellevue Hospital Medical College, New York University, and the Children's Medical Division, Bellevue Hospital, New York.

A study was made of the antigenic changes that result when milks are boiled, evaporated, superheated, dried or acidified. This was done to determine why individuals sensitive to raw or pasteurized milk can generally tolerate milks modified by heat.

The milk proteins were purified and their immunochemical reactions studied by the anaphylaxis method in the guinea pig. It was found that the proteins isolated from raw milk are chemically and biologically distinct.

The immunochemistry of evaporated, superheated, freshly boiled, acidified and dried milks and their immunological relation to the purified proteins from raw milk were studied. It was found that there were no antigenic changes as a result of drying, acidification or pasteurization. Milks that were dried, acidified, superheated or evaporated showed no loss in the antigenic properties of the casein fraction. As a sensitizing agent, given by injection, evaporated, freshly boiled and superheated milks showed practically no loss in the antigenic character of the lactalbumin fraction but as a shock agent these milks showed an unmistakable loss in the antigenic properties of the whey proteins.

Raw and the various modifications of milk described above were fed to a large number of guinea pigs. Evaporated, freshly boiled and acidified evaporated milk, when fed, showed a marked reduction in sensitizing ability. The acidified raw, dried and superheated milks did not differ from the raw milk.

A human case markedly sensitive to lactalbumin was studied immunologically. This patient developed anaphylactic shock with recovery when given 0.02 cc. of a 1% solution of pure lactalbumin intracutaneously. The blood serum containing lactalbumin antibodies passively sensitized normal guinea pigs and the skin of normal human recipients. The child was shown to tolerate evaporated milk in large amounts although raw milk, taken by mouth, in very small amounts produced anaphylactic reactions.

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* This work has been aided by a special grant from the National Research Council.

The loss in antigenic properties in heated milks is presumably due to the coagulation of the whey proteins, for which there is some evidence of reversibility. A further reduction in antigenic properties of heated milks when fed is due to the fact that heating makes for more complete digestion, and thus diminishes the probability of the absorption of native proteins through the intestinal wall.

7217 C

The Normal Polynuclear Count in Man.

JULIUS C. ABELS. (Introduced by Eric Ponder.)

From Washington Square College, New York University.

Ever since Cooke's polynuclear count became generally used in physiology and clinical medicine, the figures for the normal count in man, as given originally by Cooke and as republished by Cooke and Ponder[1] have been accepted as correct. These figures are as follows:

	I	II	III	IV	V	Mean
Lowest normal count	15	34	40	11	0	2.47
Average normal count	12	25	44	15	4	2.74
Highest normal count	9	24	47	17	3	3.11

Cooke and Ponder's normal count is based on the average of the counts for 90 people of both sexes between the ages of 12 and 55, the greatest possible care being taken to exclude foci of infection. That their standard was exceedingly rigorous is indicated by the fact that these 90 "normals" took many years to collect.

But it has been suspected that the criterion of normality used by Cooke and Ponder was too rigorous, and that the counts of "normal" people, in the sense in which the term is generally used by physiologists and clinicians, are more left-handed than the above figures indicate. Kennedy[2] has reinvestigated the matter, using a less rigorous "health standard" more likely to be met by people "normal" in the ordinary sense. His conclusion, however, is that the figures given by Cooke and Ponder are substantially correct, and that an average polynuclear count with a mean of from 2.62 to 2.72 is met in a population of normal persons, whether normality is judged by Cooke and Ponder's very rigorous criterion or by Kennedy's looser one.

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[1] Cooke and Ponder, "The Polynuclear Count." London, 1927.

[2] Kennedy, *Quar. J. Exp. Physiol.*, 1933, **22**, 377.

Such figures may be correct for the inhabitants of England (Cooke) or Scotland (Kennedy), but they are certainly not correct for the inhabitants of New York. This fact is quite well known to physiologists here, but as no figures have been published, I have carried out a series of "normal" people here, using the same "health standard" as Kennedy's. My individuals, 100 in all, were people of both sexes who to the best of their knowledge had suffered from no acute or chronic infections for several months, and whom the clinician and physiologist would ordinarily call normal. My counts are as follows:

	I	II	III	IV	V	Mean
Lowest normal count	15	59	24	1	1	2.14
Average normal count	12	54	27	6	1	2.30
Highest normal count	9	40	38	12	1	2.56

These counts are quite incompatible with those of Cooke and Ponder and of Kennedy, for my highest count is less than Kennedy's average, and my average less than Cooke and Ponder's lowest.

There are only 3 possible explanations for this difference of result. The first is that Cooke and Ponder's average count is obtained only in persons conforming to their very rigorous "health standard", and that Kennedy obtained the same sort of figure with his less rigorous "health standard" by "counting high", *i. e.*, by using Cooke's criterion for determining nuclear lobation less rigorously than did Cooke and Ponder. This possibility can be excluded because Dr. Ponder tells me that he and Kennedy, who have often worked together, count identically. The second explanation is that I "count low", *i. e.*, that I apply Cooke's criterion too rigorously. To exclude this possibility I have had my counting checked by Dr. Ponder and also by Mr. Macleod, who has worked both here and in Scotland with Ponder. The last explanation is that the average normal polynuclear count differs according to locality, being higher in England and Scotland than in New York. Considering the factors which maintain the polynuclear count in a steady state, this is not so unlikely as appears at first sight, and Mr. Macleod is at present engaged in making polynuclear counts on normal blood obtained from all parts of the world with a view to deciding the matter. The indications at present are that considerable local variations in fact exist.

7218 P

Rate of Escape of Haemoglobin from the Erythrocyte.

ERIC PONDER.

From Washington Square College, New York University.

The technical problem of finding the rate at which pigment leaves the mammalian red cell was proposed to me by Dr. Hugo Fricke, with the idea that if the rate were known it might be possible to calculate whether the pigment leaves the cell through one or two holes in the membrane, or by diffusing across the membrane generally.

The method which I have used consists in taking motion pictures of the haemolysing cells.* The best cell to work with is the red cell of man, which is relatively large. A suspension is made by suspending the thrice-washed cells from 4 cc. of blood in 20 cc. of 1% NaCl, for a suspension of this strength, when diluted with equal volumes of a lysin solution, shows from 5 to 15 cells per frame of the film used (35 mm.). Saponin made up in 1.0% NaCl in such a dilution as produces complete lysis in about 3 min. is a convenient lysin. The optical system used should be one of the highest resolution practicable, *e. g.*, an aplanatic condenser of N.A. 1.4 working at 2/3 cone and critically focussed, a 1/10 inch oil-immersion objective of N.A. 1.37, and a 10x eyepiece. If the illuminant is an arc used without color filters, the exposure is about 1/30 second. A film speed of 16 frames per sec. is quite sufficient.

To 0.5 cc. of the suspension is added 0.5 cc. of the dilution of lysin sufficient to give complete lysis in about 3 min. A drop of the mixture is rapidly placed on a slide and covered with a coverglass smeared with vaseline along the edges. The vaseline seal prevents the cells from drifting. The preparation is placed on the stage of the microscope, and the cells focussed through a side telescope which enables them to be seen while the film is in a position ready for exposure. It takes about 30 sec. to make the preparation and focus the cells. The film is then exposed until most of the cells in the field, which can be seen and kept in focus all the time through the telescope, have haemolysed.

There are two methods of dealing with the finished film, which must be developed under strictly standard conditions. The first is to look at it in a machine which drives it at such a rate as to give the sensation of continuity, to fix one's attention of a single cell, and to take the time between the moment when it first begins to fade and the time when it appears a mere shadow with a pigment concentration about 10% of the initial. Since the footage which passes through the camera as the film is being made and also the footage which passes through the machine as the film is being observed are known, one can arrive at a very good idea of the time which the cell takes to haemolyse, and the fact that the machine can be reversed so as to give the appearance of the shadows regaining haemoglobin makes the observations all the more accurate.

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* I have to thank Dr. Robert Chambers for letting me use his motion picture camera, and I have been able to avail myself of the expert technical assistance of Mr. C. Grand.

Observations made in this way show that the loss of pigment is quite a slow process, occupying from 2 to 6 sec. This is very much slower than would correspond to the haemoglobin diffusing generally across a completely permeable membrane (*i. e.,* through water). There is no visual evidence that it leaves through a rent or hole, and, although the slow rate is consistent with this mode of escape, it is equally consistent with the pigment's leaving by diffusion across an injured membrane which permits less free diffusion than does water.

The second method of dealing with the film is to carry out densitometry on the film-images in every fourth frame, so as to determine the amount of pigment in the cells at intervals of 0.25 sec. This is a highly technical procedure. It is sufficient to say that the rate of loss of pigment from the individual cell is such as might be expected if the loss were roughly represented by an exponential function. The results, which are of little interest apart from their mathematical treatment, and also results (very similar in kind) obtained when other lysins and types of cell are used, will be reported elsewhere.

7219 C

Quantitative Nature of the Red Cell Response to a Single Bleeding.

ALBERT S. GORDON. (Introduced by Eric Ponder.)
From Washington Square College, New York University.

In the course of experiments designed to test for the presence of an erythrocytogenic hormone in the serum of rabbits subjected to bleedings,[1] a close correspondence between the number of red cells and reticulocytes of several control animals during the recovery period was observed. These control rabbits were bled a certain percentage of their body weight by cardiac puncture, and red cell and reticulocyte counts made daily for approximately 2 weeks following the bleeding. Because considerable discrepancy was found between counts made on successive samples taken from the ear veins, the red cells and reticulocytes were counted in samples drawn from the heart. Care must be taken that the heart punctures are made cleanly and that the needle is held steadily while the blood is being withdrawn. When these precautions are observed, autopsies performed on animals subjected to daily heart punctures for as long as a period of a month showed no damage to the heart or hemorrhage around it. The red cell counts which were required to agree to within 2.5%, were made in duplicate, a total of 800 squares on a Levy-Hausser chamber being counted for each sample of blood. Reticulocytes were counted in wet mounts according to the method described by Ramsey and Warren.[2]

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[1] Gordon, A. S., and Dubin, M., *Am. J. Physiol.*, in press.

TABLE I.

	31		32		34		35		52	
	Wt. 3.25 kilos		Wt. 2.56 kilos		Wt. 2.85 kilos		Wt. 2.85 kilos		Wt. 2.70 kilos	
Day	R.B.C.	Ret.	R.B.C.	Ret.	R.B.C.	Ret.	R.B.C.	Ret.	R.B.C.	Ret.
1	5,218	0.9	5,470	1.0	5,322	1.1	5,724	0.9	5,268	0.3
2	3,962	2.6	3,818	2.6	3,776	2.6	4,136	1.5	4,108	1.0
3	3,592	3.9	3,728	3.1	3,612	4.3	3,862	3.5	3,580	4.9
4	3,548	4.4	3,838	5.6	3,592	5.8	3,974	5.6	3,688	6.9
5	3,550	5.1	3,842	6.1	3,572	7.7	3,904	6.4	4,036	7.6
6	3,870	7.2	4,018	7.8	3,798	8.1	4,162	7.9	4,184	6.4
7	4,092	5.0	4,006	4.7	4,116	7.4	4,324	7.6	4,152	4.8
8	3,980	4.1	4,126	4.0	4,218	5.9	4,330	4.9	4,358	3.6
9	4,116	2.8	4,320	3.2	4,008	3.6	4,750	4.5	4,224	2.4
10	4,376	1.6	4,414	1.5	4,416	2.5	4,826	2.6	4,300	2.4
13	4,614	1.2	4,572	1.1	4,512	2.1	4,914	2.8	4,622	2.0

In Table I are presented the red cell counts ($\times 10^{-3}$), and reticulocyte counts (%) for 5 animals bled 1/64 of their body weight on the first day. The reticulocyte peak, ranging from 7.2% to 8.1%, is reached in all the animals on the 4th or 5th day of anemia. There is a fairly close similarity in the behavior of the red cell counts. Some of the animals, however, showed a greater post-hemorrhagic drop (*i. e.,* that occurring after the first day of anemia) than others.

If such a relatively good quantitative picture occurs with animals picked at random, with no regard as to weight, age, breed, etc., it seemed likely that a still more quantitative set of results might be obtained if animals from the same litter were chosen. Consequently 5 litter mates were subjected to the same procedure (*i. e.,* bled 1/64 of their body weight on the first day) and counts made for a period of about a month after the bleeding. The results are given in Table II. Here the reticulocyte peak, ranging from 6.6% to 7.2%, is reached in all the animals on the 5th day of anemia. The red cell counts show a remarkably close correspondence, all the animals recovering completely from the anemia on about the 30th day after the bleeding. The same is true of the reticulocyte counts, which return to normal at approximately the same time as the red cell counts do. No post-hemorrhagic drops were observed in any of the 5 animals. All the animals, however, showed no significant increase in their red cell counts for about 6 days following the bleeding.

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[2] Ramsey, R., and Warren, C. O., Jr., *Quar. J. Exp. Physiol.*, 1932, **22**, 49.

TABLE 2.

	81		82		83		84		85	
	Wt. 1.97 kilos		Wt. 1.92 kilos		Wt. 1.87 kilos		Wt. 1.87 kilos		Wt. 2.02 kilos	
Day	R.B.C.	Ret.	R.B.C.	Ret.	R.B.C.	Ret.	R.B.C.	Ret.	R.B.C.	Ret.
1	6,198	0.7	6,044	1.0	5,620	0.7	6,262	0.3	6,256	0.2
2	4,124	1.2	4,106	1.3	3,762	1.5	4,524	0.6	4,462	0.9
3	4,284	3.2	4,220	2.1	3,704	2.6	4,684	1.6	4,426	2.5
4	4,436	4.5	4,332	3.8	3,868	4.8	4,592	3.3	4,548	4.6
5	4,408	5.7	4,344	5.4	4,024	5.8	4,560	5.3	4,466	6.4
6	4,492	6.9	4,368	7.1	3,922	6.6	4,584	7.2	4,528	6.6
7	4,522	6.2	4,352	6.4	3,964	5.6	4,612	5.6	4,568	5.0
8	4,458	4.7	4,444	3.7	4,010	4.0	4,528	4.3	4,602	3.5
9	4,602	4.1	4,520	3.3	4,108	3.7	4,504	3.9	4,516	2.8
10	4,824	3.2	4,728	3.0	4,284	3.4	4,876	3.5	4,796	2.9
12	5,012	3.1	4,840	2.7	4,442	3.3	5,122	2.9	4,910	2.2
14	5,176	2.5	5,008	1.9	4,628	2.3	5,204	2.3	5,086	1.9
17	5,286	2.0	5,174	1.8	4,852	1.8	5,402	2.3	5,198	1.6
24	6,012	1.2	5,820	1.4	5,596	0.8	6,032	1.0	6,128	1.8
31	6,108	0.6	6,086	0.8	5,712	1.5	6,214	0.4	6,212	0.6

Calculation shows that the red cell drop on the first day of anemia in these 5 animals ranges from 30% to 32%. This percentage drop is necessary to produce a reticulocyte peak of approximately 7.0%. With the first group of animals (those presented in Table I) the percentage drops on the first day of anemia do not fall within as small a range as that obtained in the second group, but if instead of computing the percentage drop on the day following the bleeding, the drop from the normal count to the lowest count in the post-hemorrhagic period is calculated, as small a variation as that observed with the litter mates is obtained (30% to 33%).

These results show that a stimulus such as a bleeding which results in a loss of approximately the same percentage of red cells, elicits a quantitative response from the bone marrow in the form of an outpouring of a remarkably constant quantity of young red cells into the circulating blood.

7220 C

Loss of Sensitivity to the Gonadotropic Hormone of the Hypophysis.

H. SELYE, J. B. COLLIP AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal.

The continued administration of the anterior pituitary-like hormone of pregnancy urine (A.P.L.) to virgin rats leads at first to a great increase in the size of the ovaries; but after some weeks the animals appear to become insensitive and in spite of continued treatment the weight of the ovaries is found to decrease to normal or even sub-normal levels. We have previously shown[1] that such "insensitive" rats will still react to gonad-stimulating extracts prepared from the hypophysis, showing renewed increase in the weight of the ovaries with definite signs of luteinization.

Eight female rats, initially 21 days old, received implants of 1-1½ rat hypophyses daily for 68 days. At the end of this period the ovaries were found, on laparotomy, to be normal or sub-normal in size. Single ovaries removed from 2 animals weighed 24 and 9 mg. respectively; one animal was sacrificed, and its ovaries were found to weigh 8.5 mg. each. The 7 survivors were then treated with 250 units of A.P.L. daily for 13 days, the implantation of hypophyses being continued. The animals were then killed, and the ovaries were found to be greatly enlarged, each ovary weighing from 119 to 198 mg. We conclude that rats treated with pituitary implants eventually lose their sensitivity to the hypophyseal gonadotropic hormone, but remain sensitive to the anterior pituitary-like hormone of pregnancy urine. The experiment may also be considered to provide further evidence that these 2 gonad-stimulating substances are not identical.

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[1] Selye, Collip and Thomson, PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487.

7221 C

Studies with Chick Embryo Tissue in Cultivation of B. Leprae.

REBIE A. HOLT. (Introduced by C. W. Duval.)

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Since the discovery by Hansen of the acid-fast bacillus in the lesion of human leprosy, many investigators who have attempted its cultivation have considered their "culture" the authentic one. Recently Soule and McKinley[1] in their attempts at cultivation of *B. leprae* prove no exception to this rule. In an earlier publication[2], they claim complete success with the CO_2, O_2 method of Wherry[3] regardless of the nutritives employed. McKinley and Verder[4] later claim that they have cultivated *B. leprae* in 5 days in chick embryo tissue suspended in Tyrode's solution under ordinary atmospheric conditions as well as CO_2 and O_2.

At the suggestion of Dr. Duval I have undertaken to repeat the work of McKinley and Verder. This paper reports results obtained upon that part of the work in which the chick embryo tissue is concerned.

The leprous tissue employed was the subcutaneous nodules and material from the socalled leprous abscess of human lepers, kindly supplied us through the courtesy of Dr. Denney of Carville, La. All the material was extremely rich in acid-fast bacilli as was evidenced upon microscopic examination of stained tissue sections and smear preparations. The Hansen microorganism of leprosy appeared chiefly in densely packed masses (globi) and small packets.

The leprous tissue was first cut into small pieces aseptically, then placed in a mortar and emulsified. A part of the emulsion was treated with a 3% NaOH solution for one hour, and immediately afterward was centrifugally washed several times in sterile Ringer's fluid. Leprous material which was not contaminated was used without treating it with NaOH. In addition, the purported culture of *B. leprae* in minced chick and Tyrode's solution which was sent to Dr. Duval by Dr. McKinley, was used in parallel experiments. Both liquid and solid media (plain neutral agar) containing the emulsified chick tissue were employed in the experiments. Ten-day-old chick embryos which had been aseptically removed from their shell and shell contents formed the basis of all culture media. The embryos were minced and suspended in Tyrode's solution, Ringer's fluid and adult chicken serum respectively. The proportion of chick embryo tissue to fluid medium was approximately 1 to 2.

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[1] Soule and McKinley, *Am. J. Trop. Med.*, 1932, **12**, 1.

[2] Soule and McKinley, *J. Am. Med. Assn.*, 1932, **98**, 361.

[3] Wherry, *J. Infec. Dis.*, 1930, **46**, 263.

[4] McKinley and Verder, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 659.

The various liquid media were inoculated with the leprous tissue emulsion. In the instance of the agar medium, the chick emulsion was spread over the slanted surface and the leprous inoculum placed or rubbed into this with a sterile platinum loop. A set of the inoculated media were incubated at 37°C. under ordinary atmospheric conditions and another set was incubated in Novy jars under CO_2, O_2 tension. The percentage mixture was 10% CO_2 and 40% O_2. After periods of 5, 7 and 30 days, the tubes incubated under ordinary atmospheric conditions were examined microscopically to determine whether proliferation of *B. leprae* had occurred. For this purpose smear-preparations were made and stained by the Ziehl-Neelsen method. The inoculated culture tubes under gaseous tension were not examined until one month after incubation.

In the determination of *B. leprae* proliferation in culture, we used the only index or guide available, namely, stained smear preparations from the original leprous emulsion before its inoculation to culture media. It is realized that this method at best is no reliable criterion of multiplication because no 2 smear-preparations from either the leprous inoculum or the "culture" will reveal the same microscopic picture, since with any 2 such preparations, one may show many times more acid-fast bacilli than the other. Furthermore, in making a smear-preparation from a "culture", one is as likely as not to take up on the platinum loop a small bit of the originally planted leprous tissue which, when smeared over the glass slide and stained, will reveal enormous numbers of acid-fast bacilli as compared to the index slide. This likely error in judging of microscopic growth in culture is all the more appreciated if we realize as Duval[5] has pointed out, that *B. leprae* multiplies in autolysing leprous tissue. One must be extremely careful not to mistake "proliferation" in tissue transplants for true *in vitro* growth of *B. leprae*. Multiplication is determined not so much upon what seems to be an increase in numbers of acid-fast bacilli as on the change in their morphology and tinctorial reaction. The new forms are longer, thicker and possess definite bipolar granules, while the original bacilli are shorter, thinner and apparently fragmented. As a rule, the new bacilli are not arranged in groups of parallel rods, nor do they occur in packets or globi as they commonly do in the lesion of human leprosy. With respect to tinctorial differences, the new bacilli are distinctly more acid-fast, *i. e.,* they stain more intensely and retain the carbolfuchsin after longer attempts at decolorization. The entire outline of the new bacilli is sharply discerned, whereas in the case of the old original forms, it is seen with difficulty.

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[5] **Duval, C. W., *J. Exp. Med.*, 1910, 12, 649.**

Lack of growth of *B. leprae in vitro* is evidenced by the fact that the bacilli remain as they were originally in the leprous tissue before culturing. They are, in comparison to the new forms, thin, indifferently stained, fragmented rods of varying lengths that lie more or less close together in parallel arrangement or they are concentrically aligned in masses known as "globi".

Morphological signs of retrograde changes and loss of viability occur for *B. leprae* in certain cultural environments. Here the bacilli, as repeatedly observed in our cultural studies, are weakly acid-fast or only partly resistant to the ordinary decolorizing agents. The "rods" lose their cell outline and the chromatin becomes more fragmented and granular. The "globi", though retaining their acid-fastness, appear as homogeneously pink, finely granular masses after treatment by the Ziehl-Neelsen method.

Comments. All cultures that had been incubated under ordinary atmospheric conditions, were examined microscopically at varying intervals over a period of one month. Particular attention was paid to the claim of McKinley and Verder that growth of *B. leprae* occurs in 5 days upon chick-embryo emulsed in Tyrode's solution. The microscopic criteria of growth used by us are given in the text.

No positive evidence of *B. leprae* proliferation was noted at any time in chick-embryo emulsified in Tyrode's solution for the various inoculums used, including the purported culture received from Dr. McKinley. On the contrary, many of the acid-fast bacilli of Hansen in this medium showed definite signs of degeneration and loss of viability after four weeks incubation at 37°C. Furthermore, negative cultural results were obtained with the inoculated chick-embryo emulsion under both ordinary atmospheric and CO_2, O_2 conditions.

In the light of our experiments chick-embryo tissue emulsified in Tyrode's solution is *per se* valueless as a nutritive in the *in vitro* cultivation of *B. leprae.*

7222 C

Extraction of Gonad Stimulating Substances of Anterior Lobe of the Hypophysis.

ARTHUR E. MEYER AND H. L. FEVOLD. (Introduced by F. L. Hisaw.)

From the Laboratory of the Chappel Foundation for Organo-Therapeutic Research, Rockford, Ill., and the Department of Zoology, University of Wisconsin.

It has been shown by Fevold, *et al.*[1, 2] that the gonad stimulating principles of the anterior lobe of the pituitary gland can be extracted almost quantitatively from powdered glands by means of 50% aqueous pyridine. On account of the difficulty in evaporation of the pyridine extract and the molesting odor, other methods of extracting have been used in the following experiments.

It was found that a solution of 6% normal butyl alcohol, or a 3% solution of amyl alcohol in water would also extract the active principles in a very efficient manner. The extract can very easily be concentrated by vacuum distillation at low temperature (below 40°C.) so it offers advantages over aqueous pyridine in this respect.

A comparison of the efficiency of the 2 solvents, 50% aqueous pyridine and 6% aqueous butanol, is given below. The procedure used was that given by Fevold, *et al.*, and was the same in both cases. One hundred grams of the dried acetone extracted pituitary gland from horses were used. The material was extracted each time for 12 hours with 700, 600, 600, 600 cc. of the respective solvent—50% pyridine or 6% butyl alcohol solution.

Each extract was then purified as follows: After evaporation *in vacuo* at a temperature below 40° to a volume of 500 cc., 4½ volumes of acetone were added. The precipitate thus obtained was emulsified in 1,500 cc. of water and 750 cc. of acetone were added. The insoluble material was re-extracted with 33% acetone for a second time. The combined 33% acetone solution was evaporated *in vacuo*. The residue was emulsified in 500 cc. water, and 70 cc. of saturated alcoholic benzoic acid were added. The material brought down with the precipitate of benzoic acid was washed with acetone to remove the benzoic acid, then extracted twice with 150 cc. of water; the aqueous extract was mixed with the filtrate from the benzoic acid and precipitated with 4½ volumes of acetone. The

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[1] Fevold, H. L., Hisaw, F. L., and Leonard, S. L., *Am. J. Physiol.*, 1931, **97**, 293.

[2] Fevold, H. L., Hisaw, F. L., Hellbaum, A., and Hertz, R., *Am. J. Physiol.*, 1933, **104**, 710.

precipitate was purified by dissolving it in 100 cc. of water. The addition of 50 cc. of acetone removed some impurities. The bulk of the active principle was then precipitated with 150 cc. of acetone. The small quantity of active material that remained in solution was precipitated with an excess of acetone, purified by fractional precipitation with acetone and dissolved together with the main fraction in 100 cc. of water.

The extracts were tested on immature female rats 22 days of age. They were injected twice daily, ¼ cc. per injection for 3 days, and the animals were autopsied on the morning of the sixth day. The results are given in Table I.

TABLE I.
Biological Assay of Preparations.
Ovarian Weights*

Amount injected cc. per rat	Ext. No. 609 mg.	Ext. No. 611 mg.
0.1	135	186
0.05	89	109
0.025	53	56
0.01	23	24

* The weights given are the average weight of the ovaries of two animals.
Ext. 609 = 50% aqueous pyridine extraction.
Ext. 611 = 6% ” butanol ”

The results show that both extracts have approximately a potency of 100 units per cc.,[2] the butanol extract being perhaps slightly more active. However, this is within the limits of variation.

Butyl alcohol itself is not a solvent for the hormone; neither is pure water a satisfactory medium of extraction. The possibility that a change of surface tension might play a rôle was considered. A 6% butyl alcohol solution in Traube's stalagmometer gave 261 drops; a 3% commercial amyl alcohol solution 225 drops, as compared with 100 drops of water. These figures are the highest obtainable with any of the lower alcohols in solutions of not more than 10%. Incidentally, an extraction carried out with a 3% amyl alcohol solution had approximately the same potency as the butanol extract.

The extracts can be filtered through Berkefeld medium filters and Chamberland L-3 filters without noticeable loss of potency.

Studies on the blood pressure in dogs showed that the pyridine extract retains a definite amount of the pressor principle of the pituitary, whereas its content in the butyl alcohol extract is insignificant.

7223 P

Presence of Oxytocic Substances in Urine During Labor.*

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Since substances of great biological significance are excreted in the urine during pregnancy, it seems possible that other substances of different biological action might be excreted during active labor. Guerin-Valmale, Loriot, and Verdeuil[1] claimed that labor urine produced contractions of the human uterus *in vivo*. They were able to induce labor in 2 patients 10 days before term by administering labor urine by proctolysis; the patients were delivered about 12 hours after the instillation. Barjaktarovic[2] used the same method in 10 cases during different months of pregnancy and concluded that to induce labor in the second half of pregnancy amounts up to 4,000 gm. of labor urine were necessary. We have, therefore, attempted to extract oxytocic substances from labor urine.

Urine was collected from women during active labor and combined until a total volume of 10 or 12 liters was obtained. (Pituitrin was not given to any case.) Thirteen such collections were made, and each was worked up separately. The samples were acidified with acetic acid and concentrated *in vacuo* at a temperature of 30-40°C. The method then used to obtain the oxytocic substance was that described by Kamm and his co-workers[3] for isolation of the active principles of the posterior lobe of the pituitary gland.

The final product was a crude material of a gummy character and colored with urinary pigments, 10 liters of labor urine yielding an average of 0.5 gm. This dried material was taken up in distilled water and dialyzed over night in a refrigerator in a parchment bag. The solution from the bag was then made neutral to litmus with

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* We wish to thank Professor Benjamin P. Watson for the clinical material; and Professors Charles C. Lieb and Michael G. Mulinos for their kind suggestions and help. We are especially indebted to Mrs. Mildred S. Adams, the nurse in charge of the delivery rooms, for her aid in collecting material. This research was aided by a grant from E. R. Squibb and Sons.

[1] Guerin-Valmale, Loriot, Verdeuil, *Bull. Soc. D'Obst. et Gyn.*, 1931, 619.

[2] Barjaktarovic, S. S., *Zbl. f. Gyn.*, 1933, **57**, 628.

[3] Kamm, O., Aldrich, T. B., Grote, I. W., Rowe, L. W., and Bugbee, E. P., *J. Am. Chem. Soc.*, 1928, **50**, 573.

sodium carbonate. The solution was tested, using about 0.4 cc. from a total volume of 25 cc. representing the original 0.5 gm., on human uterine muscle obtained from Caesarian sections at term. Twenty-five strips of muscle were used. The muscle was set up as described by Kurzrok and Lieb.[4] The result with the material from each of the 13 samples was a prompt and sustained contraction. When the solutions were tested on non-pregnant human uterine muscle (55 strips, obtained at various times of the menstrual cycle), there was no effect or a slight increase of tonus causing the normal contractions to occur with slightly more vigor. Control experiments were made, using material prepared by the same procedure from 2 10-liter samples of urine of male patients and 3 from non-pregnant women. These control materials, tested on parallel strips of muscle from the same pregnant (25) and non-pregnant (55) uteri as were used for the experimental materials, gave negative results, although a few gave slightly increased tonus.

The active material was tested by Dr. Mulinos, of the Department of Pharmacology, for its action on the water balance of the frog, and the result indicated that the substance has a pitocin-like action. The controls when tested in the same way had no significant effect.

The crude material is now being purified for clinical tests. Studies are in progress to determine how early in pregnancy these oxytocic substances appear and what effect they have on the uteri of early pregnancy.

7224 P

Prausnitz Küstner Reaction with Sera of Ragweed Hay Fever Patients to Ragweed Carbohydrate Fraction.

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In the course of a correlated series of laboratory and clinical investigations, which will later be reported upon in full detail, with carbohydrate obtained from ragweed pollen, certain results were obtained with the Prausnitz Küstner reaction (local passive transfer) which seem worth recording at this time.

In skin testing ragweed sensitive hay-fever patients to this carbohydrate material it was found that a percentage gave positive skin reactions to the carbohydrate about as large as was obtained with the whole pollen or any protein fraction thereof, and also that a percentage gave no reaction on skin tests to the carbohydrate.

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[4] Kurzrok, R., Lieb, C. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 268.

Serum for the Prausnitz Küstner reaction was taken (A) from those who showed the most marked reaction (relative to the results obtained with the pollen or other protein fractions thereof), (B) from those who showed no reaction to the carbohydrate although the results with the other ragweed preparations might be equal or thereabouts to those obtained in Group A.

With the sera of Group A it was found that the sensitized areas in the recipient would give a local reaction to the carbohydrate solution whereas with the sera of Group B no reaction was obtained.

The possibility of some peculiarity or exceeding susceptibility of the recipient was ruled out by obtaining the contrast of serum from Group A on one arm of the recipient (which gave a positive Prausnitz Küstner to the carbohydrate) and the serum from Group B (which was negative) on the other arm. This at the same time that the reactions obtained with both sera to whole pollen solution would be equal.

The age-old contention of there being a percentage of protein contaminant in what is labelled as "purified carbohydrate" and that the reaction was due to the presence of this protein fraction is ruled out by (a) the fact that positive and negative reactions to the carbohydrate are obtained with the same recipient. (b) while at the same time about equal reactions are obtained in this same recipient to both A. and B. sera against other whole pollen solutions. (c) in guinea pigs sensitized with whole pollen solution, no type of anaphylactic reaction could be obtained with the carbohydrate solution as the toxic dose, including the most sensitive, namely the Dale uterine horn method.

These contrasting results as between carbohydrate skin positive and carbohydrate skin negative hay fever individuals have been obtained repeatedly not only with the standard Prausnitz Küstner reaction but with a modification which I find has been used by others. This consisted of injecting (1) serum alone, (2) serum mixed with whole pollen solution and allowed to stand for 2½ hours, and (3) serum mixed with pollen carbohydrate solution and similarly treated as in 2.

With what one might label as carbohydrate positive (Group A above) serum, reinjection 24 hours later with whole pollen extract gives a positive reaction in sites 1 and 3 (which latter is usually somewhat smaller than that obtained in site 1). Site 2 is negative. In a duplicate set of prepared areas called respectively sites 4, 5 and 6, reinjection with ragweed carbohydrate solution gives a positive result in site 4 only. The size of the reaction in site 4 has hitherto always been a little less than that obtained in site 1.

The difference when carbohydrate negative serum is used for passive transfer is that no reaction is obtained in site 4 and usually the results in sites 1 and 3 are identical.

The solution of ragweed carbohydrate was made by dissolving 100 mg. in 50 cc. saline solution and diluting this 100 fold. 0.05 cc. was injected intradermally of all single solutions and 0.1 cc. of all mixtures.

It is not the intention to suggest that these observations prove that carbohydrate functions as a true antigen. That is not the interpretation. They do suggest that carbohydrate has its own type of specific immunological activity in a percentage of cases and that this can be demonstrated in those cases by the Prausnitz Küstner reaction.

7225

Hodgkin's Disease. I. Negative Skin Reactions to Gland Extracts.

E. M. CHAPMAN. (Introduced by Louis Dienes.)

From the Massachusetts General Hospital, Boston.

Because of the recent interest aroused by Frei[1] in demonstrating a diagnostic skin reaction to the whole extracts of glands in the virus disease lymphogranulomatosis (4th venereal disease) it was decided to observe the skin reactions on patients with Hodgkin's disease to the extracts of Hodgkin's diseased glands. Although the etiology of this disease remains obscure there are some[2] who believe it may be due to a virus. Skin reactions can be obtained with the extract of skin lesions in small pox and vaccinia, a virus disease.

The injection of filtrates of Hodgkin's glands has been used in treatment of the disease but the skin reactions to these filtrates have not been described adequately. Skin reactions to a preparation from

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[1] Frei, Wm., *Klin. Wchnschr.*, 1925, **4**, 2148.

[2] *Annales d'Anat. Path.*, 1931, **8**, 815.

Hodgkin's diseased glands were reported as negative in 3 cases by Fox and Farley.[3]

Here we are further recording the negative results obtained in 11 patients with Hodgkin's disease treated by Roentgen ray in whom the diagnosis was assured by biopsy. All but one patient showed a skin reaction of only a few millimeters, similar to that seen in normal persons. In this one the control, saline and 0.3% tricresol measured 0.5 cm. and the unheated extract measured 1.0 by 1.5 cm. at 24 hours, but after 48 hours measured 1-2 mm.

Extracts were made from the lymph nodes of an untreated case of Hodgkin's in the third month of the disease and also from a case of lymphosarcoma. The clinical course of these cases has verified the histological diagnosis. The fresh nodes were extracted with saline, centrifuged and 0.3% tricresol added to the cloudy, supernatant fluid. One-half of this was heated for an hour at 60°C. on 2 successive days. The final extract was not filtered.

7226 P

Etiology of Whooping Cough.*

G. S. SHIBLEY. (With the technical assistance of Helena Hoelscher.)

From the H. K. Cushing Laboratory of Experimental Medicine, Department of Medicine, Western Reserve University, and the Medical Service, Lakeside Hospital, Cleveland, Ohio.

Three beliefs exist in regard to the etiology of whooping cough: That the disease is caused by *H. pertussis*, that the agent is a filtrable virus, or that both play a part. *H. pertussis* is practically always present in the disease and absent in health.[1] That the organism plays a most important rôle has been shown by occasional successful transmission experiments[1, 3, 4] and by reports of effective protection with suitable *H. pertussis* vaccine.[1, 4, 5] The recent description of intranuclear inclusion bodies[6] taken together with certain other considerations has led some[1, 7] to believe that a virus plays either a

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[3] Fox, H., and Farley, D. L., *Am. J. Med. Sci.*, 1922, **163**, 313.

* Aided by a grant from the Rockefeller Foundation.

[1] Miller, J. J., *J. Am. Med. Assn.*, 1933, **100**, 681; excellent summary.

[2] Sauer, L. W., and Hambrecht, L., *Am. J. Dis. Child.*, 1929, **37**, 732.

[3] Rich, A. R., Long, P. H., Brown, J. H., Bliss, E. A., and Holt, L. E., *Science*, 1932, **76**, 330.

specific etiological rôle or, accompanied by *H. pertussis*, acts as primary infective agent. Attempted transmission experiments with filtrates have been equivocal or negative.[3, 4] Because of too recent isolation of the organism or of inoculation with filtrate shortly before[5] the few successful transmission experiments with *H. pertussis* are open to the criticism that a virus may have been present. It seemed necessary to devise experiments in which *H. pertussis* or virus effects were clearly separated. In this communication we are reporting successful whooping cought transmission by means of what we believe to be virus-free *H. pertussis*.

The newer knowledge of the phases of *H. pertussis*[8, 9] and their precise differentiation by electrophoresis[10] provided the desired approach. When first isolated *H. pertussis* differs culturally, morphologically, serologically, and electrophoretically from older laboratory cultures. Leslie and Gardner call the former, Phase I, and the variations rapidly appearing on subculture, Phases II, III, IV. It is possible[8, 10] that this change represents the well-known virulent ("S") to avirulent ("R") transition of other bacteria.

Our plan was to study, identify, and maintain this theoretically infective phase† for months, and then, when this had been done long enough to preclude the presence of a virus, to attempt transmission. Accordingly, we selected several fresh strains of *H. pertussis* of our own and from other laboratories and during several months checked them periodically for Phase I persistence. Our experimental animals were chimpanzees. In inoculating animals we attempted to reproduce natural, droplet, inhalation infection by spraying the organisms into the air about the animals' faces. Also, we added a few cc. of living cultures to their milk. This was done 3 successive days. During experiments animals were placed in rigorous isolation.

While doing the preliminary work referred to, we attempted transmission of whooping cough with a Phase IV, theoretically avirulent, strain ("A" No. 61 N. Y. B. of H.) After 2 weeks' isolation the animal was inoculated. No infection resulted. This conforms

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[4] Macdonald, H., and Macdonald, E. J., *J. Inf. Dis.*, 1933, **53**, 528.

[5] Sauer, L. W., *J. Am. Med. Assn.*, 1933, **101**, 1449.

[6] McCordock, H. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1288.

[7] Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 346.

[8] Leslie, P. H., and Gardner, A. D., *J. Hyg.*, 1931, **33**, 424.

[9] Lawson, G. McL., Thesis, 1932, Harvard School of Public Health.

[10] Shibley, G. S., and Hoelscher, H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 31.

† These studies will be the subject of another communication.

with Toomey's similar recently reported findings.[11] Six weeks later with the same animal we attempted transmission with a recently isolated Phase I strain ("No. 13," subcultured 4 times). Fifteen days later a respiratory infection developed characterized by slight nasal discharge and short hacking, occasionally paroxysmal cough. It lasted 14 days. The leucocyte count remained within normal limits. *H. pertussis* was not recovered. Whether this represented a virus cold, mild pertussis or a modified attack due to low virulence of the organism or to previous inoculation with Phase IV cultures, we cannot decide.

By October a second ape was tame enough for use. From strains under observation, we selected an organism seeming to possess all Phase I criteria (strain "Z" obtained from Dr. Hans Zinsser). The strain isolated April 20th and received May 20th, had been subcultured by us over 60 times.

After 5 weeks' isolation, the ape was inoculated with the theoretically infective and virus-free strain. On the 9th day the animal developed slight cough. Five days later it became paroxysmal, mounting to a series of 25-35 successive explosive coughs. Vomiting occasionally succeeded coughing. On the 19th day whoop, previously doubtful, definitely appeared. To this point, though cooperation was bad, we had failed to recover *H. pertussis* on cough plates though subsequently we succeeded in isolating the organism from nose cultures obtained on the 14th day. The disease was so typical clinically that we decided to sacrifice the animal. The white blood cell count on this date was 132,000, lymphocytes: 78.5% (average normal WBC 13-20,000, L. 60-70%).

At autopsy, cultures were taken from upper trachea and its bifurcation, left main bronchus, and small bronchi and bronchioles of both lower lobes. *H. pertussis* was recovered from all places cultured in large numbers. The organism was typical culturally, morphologically, and electrophoretically and agglutinated with our Phase I serum to full titer (1:20,000). A few colonies of *Streptococcus* were present on some plates. In no instance, despite painstaking search, were we able to demonstrate *H. influenzae* although we recovered it in nasal cultures taken during life.

Dr. Harry Goldblatt of the Institute of Pathology kindly performed the post mortem examination. The findings were limited to the chest and were consistent with the usual findings in pertussis. The anatomical diagnosis was acute catarrhal tracheitis, bronchitis, and bronchiolitis, both endo- and peri-, and severe acute emphysema. A typical finding was large and small mononuclear cell infiltration of subepithelial stroma and of tissues surrounding some bronchi and many bronchioles. Consolidation of the lungs was not found. No inclusion bodies were found. Although we recovered *H. pertussis* from bronchi and bronchioles, we failed to demonstrate to our complete satisfaction *H. pertussis*-like organisms embedded in the cilia.

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[11] Toomey, J. A., McClelland, J. E., and Lieder, L. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 403.

7227 P

Inhibition of Cardiac Accelerator Impulses by the Carotid Sinus.*

D. W. BRONK, L. K. FERGUSON† AND D. Y. SOLANDT.‡

From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania.

It has been shown by Hering,[1] Heymans,[2] and others that stimulation of the afferent endings in the carotid sinus by an increase in the endosinusal pressure causes a slowing of the heart. Some have held that this is entirely due to a reflex stimulation of vagal fibers while others have presented evidence to show that there is a reflex inhibition of the cardiac accelerators as well. The second explanation involving a reciprocal mechanism has generally appeared to be the more probable. In the present investigation we have studied certain aspects of this reflex by observing the sympathetic impulses to the heart while varying the pressure in the carotid sinus.

The experiments were performed on cats under nembutal anesthesia. The carotid sinus on each side was isolated and perfused by way of the common carotid and external carotid arteries. Inasmuch as the nerve supply remained intact an increase of pressure within the sinus caused an increase in the number of afferent impulses going to the medullary centers.[3] The effect of these impulses on the cardiac accelerator discharge was determined by record-

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* The expenses of this research have been in part defrayed by a grant from the Committee on Scientific Research of the American Medical Association.

† Associate in Surgery.

‡ Research associate, University of Toronto.

[1] Hering, H. E., *Die Karotissinusreflexe.* Dresden, T. Steinkopff, 1927.

[2] Heymans, C., Bouckaert, J. J., and Regniers, P. *Le Sinus Carotidien.* Paris, G. Doin, 1933.

[3] Bronk, D. W., and Stella, G., *J. Cell. and Comp. Physiol.*, 1932, **1**, 1.

ing with a vacuum tube amplifier and oscillograph the sympathetic impulses in one of the small nerves running from the stellate ganglion to the heart.

The general nature of the cardiac sympathetic activity has been described[*] as a persistent tonic discharge of impulses grouped into large waves. We now find that as the pressure within the sinus is raised this discharge decreases until at about 125 to 150 mm. Hg. there is a complete inhibition of the sympathetic impulses.

If the pressure within the sinus be maintained at such a level the period of complete inhibition lasts for some seconds, after which there is an escape with a return of the sympathetic discharge. The duration of this period of inhibition is a function of the endosinusal pressure. In one experiment it was 1 second at a pressure of 140 mm. Hg. and increased to 5 seconds at 300 mm. Hg. The escape may be due to adaptation of the endings in the sinus or to some process of adaptation in the centers. This latter explanation seems more probable because the pressure endings in the carotid sinus adapt slowly and relatively little, but a definite conclusion must wait on further experiments.

It is generally stated that the slowing of the heart caused by an increased pressure in the carotid sinus is due to a unilateral reflex. Such an opinion is founded on experiments which showed no decrease in heart rate when the vagus, on the side of the sinus which was stimulated, was cut, even though the other vagus was intact. Our experiments, on the other hand, indicate that the reflex inhibition of accelerator tone is bilateral. Distension of either carotid sinus produces inhibition of the cardiac sympathetic impulses from both stellate ganglia.

In keeping with this fact is our finding that the duration of the sympathetic inhibition resulting from the simultaneous distension of both carotid sinuses is equal to the sum of the periods of inhibition resulting from the distension of each sinus separately. There is very definite evidence of summation.

The duration of accelerator inhibition resulting from a given degree of carotid sinus stimulation is often increased by section of both aortic nerves. This fact is related to Hering's observation that one carotid sinus is more effective in lowering blood pressure if the other sinus is denervated and the aortic nerves are cut.

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[*] Bronk, D. W., and Ferguson, L. K., PROC. SOC. EXP. BIOL. AND MED., 1932, 30, 339.

7228 C

A Color Reaction for Carotene.

VICTOR E. LEVINE AND GEORGE E. BIEN.

From the Department of Biological Chemistry and Nutrition, School of Medicine, Creighton University, Omaha, Nebraska.

Carotene in chloroform solution reacts with formaldehyde in sulphuric acid to form a characteristic deep violet zone between the chloroform and acid layer. The violet zone on shaking disseminates in the acid giving rise to a violet colored lower layer, while the chloroform layer above remains colorless. The addition of 2 to 3 drops of acetic anhydride causes a transitory blue to appear in the acid layer, provided a high concentration of carotene is present in the chloroform solution.

The test, which is considered positive on the appearance of a characteristic deep violet zone, is very sensitive, serving to detect 0.01 mg. of carotene in 2.5 cc. of chloroform. To carry out the test equal volumes of chloroform and formaldehyde-sulphuric acid mixture should be employed. For convenience we have used 2.5 cc. of the chloroform solution with 2.5 cc. of the aldehyde-acid mixture. This mixture is made up in the proportion of 1 volume of 37 to 40% formaldehyde to 50 volumes of concentrated sulphuric acid. Both the formaldehyde and the sulphuric acid should be chemically pure, and the mixture freshly made before using.

Cholesterol, according to Whitby,[1] gives a characteristic reaction with the formaldehyde-sulphuric acid mixture described above. With 1 to 2 mg. of the sterol dissolved in 2 cc. of chloroform he reported a cherry red color in the upper chloroform layer and a brownish red color in the lower sulphuric acid layer, which also showed an intense green fluorescence. When 2 to 3 drops of acetic anhydride were added to the chloroform layer previously transferred to a dry test tube, a blue color formed. With 0.1 mg. of cholesterol in 2 cc. of chloroform he obtained a deep reddish brown color and a very marked fluorescence in the acid layer, but no color in the chloroform layer. With 0.01 mg. of the sterol he could develop only a faint fluorescence in the acid layer and no coloration in the chloroform layer.

With 0.1 mg. of cholesterol in 2.5 cc. of chloroform we observed with the Whitby procedure a brown color and green fluorescence in the acid layer, but no coloration in the chloroform layer. With 0.2 mg. cholesterol in 2.5 cc. chloroform we were able to observe a faint pink in the chloroform layer. From the standpoint of color formation in the upper chloroform layers, we may state that cholesterol is not as sensitive as carotene to the formaldehyde-sulphuric acid reaction.

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[1] **Whitby, G. S., *Biochem. J.*, 1923, 17, 5.**

Using Salkowski's procedure for the qualitative detection of cholesterol, we obtained an indigo blue color with a chloroform solution of carotene to which concentrated sulphuric acid had been added. This indigo blue also develops when crystals of carotene are dissolved in concentrated sulphuric acid. We are therefore led to the belief that the violet zone produced with a chloroform solution of carotene on the addition of formaldehyde-sulphuric acid mixture represents a blending of the blue color arising in the presence of the acid with a red shade probably formed by the intervention of the formaldehyde.

The copper-colored crystals of carotene turn pale yellowish brown on exposure to air and sunlight. The oxidized carotene does not respond to the formaldehyde-sulphuric acid reagent in the characteristic way, developing a brown zone instead of one which is deep violet.

7229 C

Liebermann-Burchard Reaction with Compounds Containing Five-Membered Monoheterocyclic Rings.

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Sterols,[1] terpenes,[2] carotene,[3] vitamin A[4] and 5-membered monoheterocyclic ring compounds[5] have the characteristic in common that they react with the Carr-Price reagent, antimony trichloride in chloroform solution, and with the Shear reagent, aniline-hydrochloric acid.[6] Reasoning on the probability of the existence of some common factor in the chemistry of sterols, terpenes, carotene and vitamin A, we determined to find out whether the Liebermann-Burchard reagent, used qualitatively in the detection of cholesterol and quantitatively in its estimation, was also given by the other compounds in the group we have mentioned.

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[1] Wokes, A. F., *Biochem. J.*, 1928, **22**, 830; Heilbron, M., and Spring, F. S., *Biochem. J.*, 1930, **24**, 133; Seel, H., *Arch. ex. Path. u. Pharmakol.*, 1931, **159**, 92.

[2] Levine, V. E., and Richman, E., *Biochem. J.*, 1933, **27**, No. 6, in press.

[3] Moore, T., *Lancet*, 1929, **1**, 499; von Euler, B., von Euler, H., and Hellström, H., *Biochem. Z.*, 1928, **220**, 370; Karrer, P., *Bull. Soc. Chim.*, 1928, **43**, 1041.

[4] Carr, F., and Price, T. A., *Lancet*, 1929, **2**, 806; Willimott, B. G., and Wokes, F., *Lancet*, 1927, **2**, 8.

[5] Levine, V. E., and Richman, E., *J. Biol. Chem.*, 1933, **101**, 373.

We accordingly studied the Liebermann-Burchard test with respect to certain 5-membered monoheterocyclic compounds. Unless otherwise stated, we adopted the procedure of dissolving 1 to 2 drops of a given liquid compound to be tested or 10 to 20 mg. of a solid compound in 2 cc. of chloroform, adding 10 drops of acetic anhydride and 1 to 3 drops of concentrated sulphuric acid. The color reaction undergoes many variations depending upon the concentration in chloroform of the compound tested and the quantity of concentrated sulphuric acid added.

Pyrrol at first gave a yellow turbidity, which gradually turned to very deep orange. On standing a deep blood-orange precipitate separated out, leaving a colorless liquid above. On the addition of sulphuric acid alone slightly heavy droplets separate at the bottom. On standing these droplets solidified and developed an orange tinge, which gradually darkened. Thiophene on the addition of 5 drops to 2 cc. of chloroform gave yellow oily droplets at the bottom of the tube. On standing over night the droplets disappeared and the liquid contents of the tube turned green. The reaction was very slow. The green color, however, once formed became deeper and lasted more than 2 weeks. Black granules finally precipitated out in the colored mixture. With sulphuric acid alone yellow droplets formed at the bottom. These gradually turned deep reddish brown, and at the end of a week the colorless liquid assumed a very slight green tinge.

With furfurane a green viscid precipitate formed, which on standing dissolved to form a deep grass-green solution, turning violet and subsequently deep blue, and finally returning to deep grass-green. The reaction with furfurane is very characteristic and very intense. The final color is very stable and remained for more than a week. With sulphuric acid alone, a red-brown gummy precipitate developed, the liquid reaction mixture remaining colorless. With dimethyl furfurane yellowish droplets were formed, clinging to the sides of the tube in a colorless liquid reaction mixture. The droplets changed to deep orange, and finally the colorless liquid assumed a very deep orange, which changed to deep cherry-red. With sulphuric acid alone yellow, oily droplets formed, which finally turned brown.

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[6] Levine, V. E., and Seaman, C. L., *Biochem. J.*, 1933, **27**, No. 6, in press; Levine, V. E., and Shaughnessy, E. J., *Biochem. J.*, 1933, **27**, No. 6, in press.

The Liebermann-Burchard reagent also reacted with other derivatives of furfurane. Furfuryl alcohol gave heavy black precipitate and an intense black liquid. On dilution with 1 to 2 volumes of chloroform, the reaction mixture showed up a green color. Tetrahydrofurfuryl alcohol in chloroform solution after the addition of acetic anhydride developed a green color with 1 drop of sulphuric acid.

Furfural developed a succession of colors ranging from yellow to green to cherry-red, to brown, to purple and finally to deep green. Furfuraldoxime gave a green-yellow color when acetic anhydride alone was added. On the addition of 1 drop of sulphuric acid, the color changed to reddish yellow and finally became reddish brown. Furfural acetone yielded a yellowish brown color which, on the addition of an extra drop of sulphuric acid turned green. Furfural acetophenone produced a greenish yellow, changing to red and finally to brown; furfural diacetate, a cherry-red. Furylacrolein after the addition of 1 drop of sulphuric acid formed a yellow color which changed to green. On the addition of another drop of acid, the color became intense blue. Furylacrolein oxime gave a deep chlorophyll green coloration.

Hydrofuramide turned brown with a greenish tinge. On dilution of the reaction mixture with an equal volume of chloroform the color turned decidedly green with an olive tinge. Both furil and furoin gave the characteristic green color reaction, which is similar to that obtained with cholesterol.

Furoic acid and furoamide displayed no reactivity. The latter is insoluble in chloroform. Furonitrile, dissolving very slowly, yielded a colorless mixture at first, later changing to olive-green. Furylacrylic acid gave a bright red solution which changed to purple and finally to green. Furylacrolyl chloride yielded droplets which eventually turned green. Furylacrylamide produced a yellowish coloration which gradually became green.

We are led to the conclusion that the Liebermann-Burchard reagent not only reacts with cholesterol, but also with thiophene and with furfurane and many of its derivatives.

7230 P

Results of Prolonged Increased Iodine Feeding.

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The advisability of adding iodine to the diet of man and animals has long been questioned. The herd of Brown Swiss dairy cows at the farms of Mr. William Wallace Kincaid,* Ellerslie-on-Niagara, New York, had been fed daily large amounts of iodine during a period of 2 years and 11 months. They were, consequently, satisfactory experimental animals for investigating the effects of prolonged increased iodine feeding. The blood iodine was of particular interest. The normal bovine blood iodine is 13,[1] or 14[2] gamma %. In 19 cows of this herd the blood iodine ranged from 35.9 to 78.1 gamma %, averaging 55.

Analyses of the feed given these cows daily, revealed the increased iodine intake (Table I). Since the high milk producing cows were fed more of the "grain mix" they ingested greater amounts of iodine (Table I, a). The natural iodine content of the "grain mix" had been increased by the addition of potassium iodide, fish meal, and kelp. The iodine content of this thoroughly mixed food was 2.006 mg. per 100 gm. The daily iodine intake of the cows varied from 68.6 to 196.1 mg.

TABLE I.
Daily Iodine Intake.

Source	I. Mg. %	Kg. Fed Daily	Mg. I. Fed Daily
"Grain Mix"	2.0060	2.721 to 9.071 (a)	54.59 to 181.98 (a)
Alfalfa Hay	.0326	11.339	3.70
Carrots	.0099	9.072	0.90
Wet Steamed Feed	.0508	9.072	4.91
Ensilage	.0391	10.886	4.26
Water	.0007	31.751 to 45.359	0.23 to 0.33

Large amounts of iodine, from 51.7 to 163.4 mg. daily, were eliminated, Table II. More was eliminated in the urine than in either the milk or the feces. The high milk producing cows likewise showed the greater elimination of iodine, (Table II, a).

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* We express our appreciation of Mr. Kincaid's generous cooperation.

[1] Kendall, E. C., *J. Biol. Chem.*, 1920, **43**, 149.

[2] Phillips, F. J., and Curtis, G. M., unpublished studies.

TABLE II.
Daily Iodine Output.

Origin	Output	I. Gamma %	Mg. I.
Urine	10.1 to 18.6 l.	269 to 1005	34.6 to 130.0 (a)
Feces	9.1 to 29.5 k.	140 to 468	15.2 to 71.5 (a)
Milk	7.7 to 27.5 l.	15.7 to 181.9	1.5 to 19.5 (a)

The milk iodine was increased. It ranged from 15.7 to 181.9 gamma per 100 cc., averaging 66.4. The average iodine content of milk from cows on usual feed is 5 gamma per 100 cc.[2, 3]

All physical examinations showed the cows to be in very good general condition. Cows of the herd previously sterile have become pregnant and borne normal calves during this regimen of feeding. The calves developed normally. No objective symptoms of abnormalities were discerned in the entire herd. There was no evidence of hyperthyroidism. The milk and butter fat production of the entire herd has definitely increased during this period of increased iodine feeding. During the year 1932 one of these cows became one of the highest milk producers of the breed.†

7231 C

Scalp Products and Hair as a Culture Medium for Certain Pathogenic Fungi.

JOHN W. WILLIAMS.

From the Homberg Memorial Infirmary, Massachusetts Institute of Technology.

In the present work observations were made of the growth of certain pathogenic fungi on hair. Bonar and Dreyer[1] used bundles of hair inoculated with trichophyton interdigitale to test antiseptic properties of solutions. Tona[2] observed the growth of trichophyton on hair in the test tube. The growth of the various pathogenic fungi (dermatophytes) on a medium of scalp products and hair seems to have received little attention.

The medium used consisted of barber shop hair cleansed with water, placed in petri dishes and test tubes and autoclaved. It was

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[3] Schwaibold, J., and Scharrer, K., *Biochem. Z.*, 1927, **180**, 334.

† Milk—21, 924.9 lb., butter fat—1,037.1 lb., % butter fat—4.73, for 1932, by G. V. L. No. 18823.

[1] Bonar, Lee, and Dreyer, A. D., *Am. J. Pub. Health*, 1932, **22**, 909.

[2] Tona, A., *Ann. de dermat. et syph.*, 1928, **10**, 194.

found necessary to keep the medium moist with distilled water by adding an amount sufficient to cover the mat of hair. Growth on this medium was compared with that on conservation Sabouraud's. Sabouraud's conservation medium was chosen as a standard because of its simplicity. Bacto products were used in making the media.

Transplants were made from 30-day-old growths of the organisms on Sabouraud's proof agar incubated at room temperature.

Approximate time of growth of the following pathogenic fungi and the 2 saprophytes, *Lichtheimia sp.* and *Scopulariopsis brevicaulis* were noted: *Achorion schoenleinii, Acladium castellani, Candida candida, Endodermophyton tropicale, Endomyces capsultus, Endomyces dermatitidus, Epidermophyton inguinale, Glenospora gammeli, Geotrichum bachmann, Indiella americana, Monilia albicans, Microsporon apiospermum, Microsporon audouini, Oöspora humi, Trichophyton crateriforme, Trichophyton granulosum, Trichophyton japonicum, Trichophyton interdigitale, Willia anomala.* Observations were limited to 30 days. Nine organisms which grew well on Sabouraud's medium showed no growth on water hair; *Achorion schoenleini, Acladium castellani, Candida candida, Endomyces dermatitidus, Epidermophyton inguinale, Glenospora gammeli, Microsporon audouini, Oöspora humi,* and *Willia anomala.* Since the pathogenicity of the *Microsporon audouini* manifests itself before puberty its growth was observed on a medium of child's hair. Growth was noted in 7 days as contrasted to more than 30 days on the barber shop water hair medium. In the majority of instances growth on conservation Sabouraud's medium occurred more rapidly than on water hair medium. In both, growth within 4 days was noted with *Indiella americana, Lichtheimia sp., Microsporon apiospermum, Scopulariopsis brevicaulis, Trichophyton japonicum* and *Trichophyton interdigitale.* Growth was noted within 4 days on the conservation Sabouraud's and within 7 days on the water hair with *Geotrichum bachmann* and *Trichophyton granulosum.* Growth was noted within 4 days on the conservation Sabouraud's and within 20 days on the water hair with *Endomyces capsulatus, Monilia albicans* and *Trichophyton crateriform.* Growth was noted within 7 days on the conservation Sabouraud's and within 5 days on the water hair with *Endodermophyton tropicale.* Although the number of pathogenic fungi growing on water hair medium seems limited and their growth in many instances is slow, importance is attached to this observation since it is possible to visualize human scalps as possible foci of these organisms with or without resultant local symptomatic manifestations.

Ability of certain of these organisms to grow on hair should prove a point of differentiation. Determination of the growth factor will necessitate the separation of the hairs from the desquamated epithelial scales and the secretions of the scalp. This work is now in progress.

7232 P

Reduction of Methylene Blue by the Blood of Young Infants.

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The blood of infants shows significant alterations in the first few months of life. Most pronounced, perhaps, is the drop in the number of red blood cells and in hemoglobin. Another hitherto unrecognized feature apparently characteristic of this period is the rapidity with which the blood decolorizes methylene-blue.

The method employed in this investigation was as follows: A stock solution was prepared by dissolving 200 mg. of methylene blue* in 40 cc. of 3% sodium citrate* in doubly distilled water at pH 7.4 to 8.1. After filtration 0.07 cc. of this solution was dissolved in 50 cc. of sodium citrate of the same strength, giving a final concentration of methylene blue of approximately 1:140,000. This solution was left at least 24 hours in the ice-box before use. For each test 4 samples of freshly drawn blood was added to 0.1 cc. of the methylene blue solution. The quantities of blood with youngest subjects were .02, .03, .04, and .05 cc. respectively, and a range of .03 to .06 cc. in the older ones. The contents were thoroughly admixed and kept at 15°C. for 1 hour. The tubes were then centrifuged for 2 minutes. The extent of reduction was 0 when the dilute blue color was unchanged, to 4 plus when the dye was completely reduced to that of water.

This study included the blood of 238 healthy individuals, 152 under one year of age, the remainder ranged to 40 years. The results are summarized in Table I.

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* Methylene Blue, U. S. P. Medicinal, National Aniline and Chemical Co. Sodium Citrate, Baker's Analyzed.

TABLE I.
Reduction of Methylene Blue by Blood of Young Infants in % Cases.

Age	No. Subjects	Degree of Decolorization					No. Observations
		0	1	2	3	4	
0-1 mo.	54	0.9	5.7	4.8	21.1	67.3	104
1-4 mos.	134	0.	1.4	4.3	7.3	86.8	205
4-8 mos.	105	33.8	19.0	16.5	13.2	17.3	121
8 mos.-1 yr.	63	59.7	22.2	12.5	5.5	0	72
1-40 yrs.	92	86.6	10.2	2.3	0.7	0	127

The reduction of methylene blue proceeded most rapidly and completely in the large majority of observations during the first 4 months of life. After this period, the reducing power was markedly diminished and resembled the blood of the older child and adult.

Tests were also carried out periodically with the blood of 3 young rabbits. The results corresponded with the human infant except that the reducing power of the animals disappeared between 2 and 3 months of age.

Following the initial readings (primary reduction phase) the tubes were placed in the ice-box (5-6° C.). Reduction of the dye proceeded with all bloods regardless of age, and in 24 hours reduction was usually complete. The more rapid rate of decolorization, however, occurred with the smallest quantities of blood in the young baby. The clearness of the supernatant layer of the young baby's blood always distinguishes it from that of older ages. After reduction had been completed in the ice-box, the clear supernatant fluid was replaced by 0.1 cc. of fresh methylene blue. After gentle shaking and sedimentation, reduction proceeded rapidly in the blood of the young infant and slowly or not at all in the blood of older individuals. Preliminary washing of the red cells by prolonged centrifugalization interfered with the reaction. The dye was not decolorized in an acid solution of a pH 6.3. No decolorization took place at 2°. The optimal temperature appeared to be 15° C. At 37° the reaction was less marked and often confused by hemolysis. At 5° decolorization persisted indefinitely. The blue color returned by exposure to higher temperatures.

The serum, plasma, platelets and white cells were eliminated as essential factors. The reduction of the dye bore no quantitative relationship to the amount of hemoglobin in the specimen or to the hematocrit volume. Directly after birth when the hemoglobin was highest the reduction of methylene blue was often much less marked than in the weeks that followed (Table I). That hemoglobin or some other portion of the erythrocyte appeared to be involved in the decolorization process, was indicated by oxidizing hemoglobin to methemoglobin by a trace of sodium nitrite. This produced methemoglobin without laking the blood and interfered with the decolorization reaction. Age of the red cells as indicated by reticulation was also eliminated as a factor since reticulated cells are present in greatest number at birth.

Since methylene blue is converted into its leuco-derivative by reduction, restoration of the color should be accomplished by oxidation. The clear supernatant layer, however, could not be oxidized chemically or by vigorous shaking to the original blue color. On the other hand, the dye was associated with the cellular layer for a blue color developed in the supernatant layer, after the addition of dye-free sodium citrate to the centrifuged cells at 37° C.

These studies show that the red blood cells of the young infant and animal possess the property of reacting with methylene blue which is modified during growth. Many of the phases of this reaction suggest a relationship to the adsorption phenomenon.

7233 P

Bacterial Variation in Pneumococcus and Streptococcus Hemolyticus.

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Arkwright[1] originally described 2 variant forms of members of the colon-typhoid-dysentery group which he designated by the terms S (smooth) and R (rough). Recently evidence has accumulated[2] to show that members of the colon-typhoid-dysentery group, as well as many other bacterial species, possess a third and distinct variant form, the M (mucoid). Organisms in the mucoid phase possess distinct capsules and form large mucoid colonies.

Griffith[3] described 2 variant forms of pneumococcus and, adopting Arkwright's terminology, designated them as S (smooth) and R (rough). A new variant form of pneumococcus was recently described by the author.[4] This new variant form was shown to be strikingly different from the S and R forms described by Griffith.

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[1] Arkwright, J. A., *J. Path. and Bact.*, 1921, **24**, 36.

[2] Hadley, P., personal communication.

[3] Griffith, F., *Rep. Pub. Health and Med. Subj., Ministry of Health,* No. 18, 1923, 1.

[4] Dawson, M. H., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 806.

A comparison of the characteristic features of the 3 chief variant forms of members of the colon-typhoid-dysentery, etc., group on the one hand, and of pneumococcus on the other hand, reveals a striking parallelism. Thus, (1) the M (mucoid) form of the colon-typhoid-dysentery, etc., group corresponds with the S (smooth) form of pneumococcus; (2) the S (smooth) form of the colon-typhoid-dysentery, etc., group corresponds with the R (rough) form of pneumococcus; and (3) the R (rough) form of the colon-typhoid-dysentery group corresponds with the newly described variant form of pneumococcus.

The striking inconsistency in terminology employed to describe corresponding variant forms of different bacterial species is obviously confusing and should be rectified if possible. Before proposing a radical change in the currently accepted terminology of pneumococcal variants a study of bacterial variation in *Streptococcus hemolyticus* was undertaken in order to determine whether similar variant forms could be demonstrated in this species.

Variant forms of *Streptococcus hemolyticus* have been described by various workers (Cowan,[5] Todd,[6] Eagles,[7] Andrewes,[8] Delves,[9] Cooley,[10] etc.) but a parallelism in the dissociative behavior of this organism and that of pneumococcus has not been demonstrated.

Isolated colonies of 7 strains of *Streptococcus hemolyticus* were allowed to age at 37° C. on blood-agar plates for periods of one to 2 weeks. At the end of this period the roughest marginal areas were picked and transferred to fresh blood-agar plates. This process was repeated several times and finally resulted in the development of extremely rough colonies which closely resembled those described as characteristic for the new variant of pneumococcus. The morphology of the individual organisms and their manner of growth in liquid media also corresponded closely with that of the newly described variant of pneumococcus. It therefore appears that *Streptococcus hemolyticus* produces a variant form which corresponds with the newly described variant form of pneumococcus.

If the phenomenon of bacterial variation in *Streptococcus hemo-*

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[5] Cowan, Mary L., *Brit. J. Exp. Path.*, 1922, **3**, 187; 1923, **4**, 241; 1924, **5** 226.

[6] Todd, E. W., *Brit. J. Exp. Path.*, 1927, **8**, 289; 1928, **9**, 1.

[7] Eagles, G. H., *Brit. J. Exp. Path.*, 1928, **9**, 300.

[8] Andrewes, F. W., and Christie, Ethel M., *Med. Res. Council*, 1931, Specia Series, 169.

[9] Delves, Edna, *J. Infect. Dis.*, 1932, **50**, 350.

[10] Cooley, L. E., *J. Infect. Dis.*, 1932, **50**, 358.

lyticus corresponds with that exhibited by pneumococcus mucoid and capsulated phases of *Streptococcus hemolyticus* should also be demonstrable. Numerous references to the occurrence of capsulated and mucoid forms of *Streptococcus hemolyticus* occur in the literature. Observations recently made in this laboratory have shown that mucoid variants of *Streptococcus hemolyticus* occur much more frequently than had heretofore been supposed. It has been shown that freshly isolated strains of *Streptococcus hemolyticus* grown on neo-peptone blood agar plates frequently produce large mucoid colonies. The peritoneal exudates of mice injected with such cultures frequently reveal the presence of capsulated organisms. Over 50 such strains have recently been identified by the author. Furthermore, mucoid forms have been obtained from non-mucoid (smooth or "glossy") stock cultures by growth on neopeptone blood agar plates under suitable conditions. Further studies on the virulence and specificity of the mucoid form of *Streptococcus hemolyticus* and the relationship which it bears to the "matt" form of Todd will shortly be reported.

In conclusion evidence has been presented which suggests that *Streptococcus hemolyticus* possesses 3 chief variant forms, which correspond closely with the 3 chief variant forms of Pneumococcus. Furthermore, these 3 chief variant forms of *Streptococcus hemolyticus* and pneumococcus correspond closely with the 3 chief variant forms of a wide variety of other bacterial species. However, at the present time there exists a striking inconsistency in the terminology employed to describe 2 of the 3 chief variant forms of pneumococcus. The third chief variant form of pneumococcus has not yet received an appropriate designation.

Evidence is rapidly accumulating to show that the phenomenon of bacterial variation in a wide variety of bacterial species fits into a more or less orderly pattern. The adoption of a uniform terminology to describe the chief phases in that general pattern would assist in the further study of the many problems in bacterial variation that await solution.

7234 P

Changes in Hypophysis of Adult Male and Female Rats After Pregnancy Urine Extract Injections.*

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Meyer, Leonard, Hisaw and Martin[1] demonstrated that oestrin diminishes the gonad stimulating capacity of the anterior pituitary. Inasmuch as the injection of P. U. extract into normal females increases their oestrin output, the potency of their pituitaries should thereby be diminished. Leonard[2] injected a large series of adult male and female rats with P. U. extracts† and subsequently tested their pituitaries for gonad stimulating potency by implantation into immature female mice. Pituitaries of the injected animals showed a marked decrease in gonad stimulating potency. The potency of the castrate's pituitary was not diminished by P. U. injections.

The pituitaries of 32 normal and castrate controls and injected rats, from the experiments of Dr. Leonard, were utilized for cytological study. They were fixed and stained by methods previously described.[3] As expected, marked changes were observed in the basophiles.[4] These cells in large part were depleted of their basophilic granules. Their cytoplasm appeared as clumped, yellowish masses frequently with small basophilic granular areas interspersed. The distinguishing Golgi apparatus was hypertrophied. The cytological picture suggests a cell which has been in active secretory phase. These basophiles range in size from normal basophiles down to cells little larger than chromophobes. An even more striking feature, however, is their obvious numerical increase. There is a corresponding decrease in chromophobes. If changes occur in the acidophiles, they are of such character that they could be detected only by careful statistical analyses of cell size and numbers. Technical meth-

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council, administered by Dr. P. E. Smith.

[1] Meyer, R. K., Leonard, S. L., Hisaw, R. L., and Martin, S. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 702; *Endocrinology*, 1932, **16**, 655.

[2] Leonard, S. L., *Anat. Rec.*, 1933, **57**, 45.

† Follutein kindly furnished by Dr. J. A. Morrell, E. R. Squibb and Sons.

[3] Severinghaus, A. E., *Anat. Rec.*, 1932, **53**, 1.

[4] Compare findings of Wolf, J. M., *et al.*, on Reaction of Anterior Hypophysis of Immature Female Rats to Placental Hormone. PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1092.

ods which do not demonstrate the Golgi region either in positive or negative form would make difficult an accurate separation of the chromophobes from the smaller of the atypical degranulated basophiles. The injections of P. U. extract, therefore, seem to cause both an increase of basophiles and a rapid atypical degranulation of their cytoplasm. The pituitaries of castrates injected with P. U. extract cannot be distinguished histologically from the castrate controls.

It was suspected during the course of the study that both the pars anterior and the pars intermedia were enlarged. Pituitary reconstructions were made on 7 adult male rats from 2 series of experiments of Dr. P. E. Smith, in which 25 R.U. of Antuitrin S daily for 75 days, and 100 R.U. daily for 71 days respectively were injected. The pituitaries from these animals were cut serially at 7µ and the pars anterior and pars intermedia were measured with a polar planimeter, every third section being measured. The results are shown in Table I. Both the pars anterior and pars intermedia are definitely enlarged. Although the pars anterior of the heaviest injected animal (9504) is slightly smaller than those of the other injected forms,[5] the pars intermedia is noticeably the largest of the group. No mitoses are found and in a preliminary study, a unit area of pars intermedia from an injected animal was found to contain only two-thirds as many nuclei as are to be found in the gland of a normal control. This indicates that the pars intermedia enlargement is due to a hypertrophy and not a hyperplasia of the cells. The involvement of pars intermedia in pituitary-gonad endocrine relations is unusual, and these findings are being subjected to much more detailed analysis.

TABLE I.
Results of Antuitrin S† Injections into Adult Male Rats.

Rat		Treatment		Weights in gm.		Relative Polar Planimeter Values		
No.	Wts. gm.	R.U.	Days	Accessories	Testes	Pars Anterior	Pars Intermedia	Total P.A. + P.I.
9505	310	Control		2.4	2.17	12306	1662	13968
9507	280	,,		2.07	1.95	11764	(1964)*	13728
9431	290	,,		2.3	1.9	12740	1656	14396
9433	286	25 (¼ cc.)	75	4.05	2.5	14207	1876	16083
9434	290	25 (¼ cc.)	75	5.2	2.34	14248	1990	16238
9435	280	25 (¼ cc.)	75	5.4	3.05	14250	1946	16196
9504	330	100 (1 cc.)	71	4.5	3.2	13912	2241	16153
9436	280	25 (¼ cc.)	75	5.4	2.8	Reserved for cytological study		

* A massive colloid cyst in the pars intermedia and extensive cellular anastomoses with the pars anterior may be factors in this abnormally large size.

† Kindly furnished by Dr. Oliver Kamm, Parke, Davis & Co.

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[5] See Collip, J. B., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 590.

7235 P

Chemical Studies in Bacterial Agglutination.*

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I. A micro method for the quantitative estimation of agglutinins.

The agglutination of bacteria may be regarded as a precipitin reaction at the surface of the cells.[1, 2] A method had been worked out in this laboratory for the quantitative estimation of precipitins,[3] and it has been found possible to adapt the procedure to the micro determination of agglutinins. Thoroughly washed, heavy bacterial suspensions are standardized by measuring their nitrogen content per cc. by the micro-Kjeldahl method. Accurately measured quantities of bacteria and an appropriate antibody solution or serum are then mixed and allowed to stand, with occasional mixing, at 37° for 2 hours and overnight in the ice-box. The remainder of the analysis is carried out as in the precipitin reaction,[3] except that it has been found convenient to make use of the higher speed possible with the angle centrifuge.† This was run in an ice-box compartment. Agglutinin nitrogen is determined by subtracting from the total nitrogen of the washed precipitate the amount of nitrogen contained in the bacteria used. Agglutinin N may also be determined from the difference between a blank without bacteria, and the supernatant from the agglutination. Both methods are illustrated in Table I. Agglutinin = agglutinin N × 6.25, under the assumption that agglutinin is protein. If the relative amounts of bacteria and serum are so chosen that the supernatant contains no more agglutinin the result obtained (divided by the volume) is the agglutinin content of the serum in milligrams per cc. In the table are given typical data for pneumococcus agglutinins. Application of the method to other systems is in progress.

An immediate outcome of the method is given in the following

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* The work described in this communication was carried out under the Harkness Research Fund of the Presbyterian Hospital.

[1] For a review, *cf.* Wells, H. G., Chemical Aspects of Immunity, 2nd Edition, New York 1929.

[2] Francis, T., Jr., *J. Exp. Med.*, 1932, **55**, 55.

[3] Heidelberger, M., Sia, R. H. P., and Kendall, F. E., *J. Exp. Med.*, 1930, **52**, 477; Heidelberger, M., Kendall, F. E., and Soo Hoo, C. M., *Ibid.*, 1933, **58**, 137.

† Furnished by the Standard Scientific Supply Company, New York.

TABLE I.

Vol. bacterial suspension	Vol. antibody sol'n	Supernatant			Aggl. N per cc. antibody sol'n	Precipitates			Aggl. N per cc. antibody sol'n
		Original N content	N after agglutination	Agglutinin N found		Total N found	N of bacteria	Aggl. N found	
cc.	cc.	mg.	mg.	mg.	mg./cc.	mg.	mg.	mg.	mg./cc.
Pn I S	B75* (Dilution 8:25)								
Susp. B									
0	1.0	.87							
3.0	0	.01					.42		
3.0	0.5†	.44	.15	.29	.58	.67	.42	.25	.50
Susp. C									
2.0	0	.02					.33		
2.0	0.5	.44	.15	.29	.58	.61	.33	.28	.56
2.0	1.0	.87	.38	.49		.80	.33	.47	
2.0	1.5	1.31	.74	.57		.90	.33	.57	
	Anti-Egg-albumin serum								
1.0	2.0					.16	.17	.00	.00
PnII-R. susp.	Anti-"R" protein serum‡								
2.0	0						.34		
	Serum 231								
2.0	0.5					.37	.34	.03	.06
	Serum 235								
2.0	0.5					.37	.34	.03	.06
	Supernatant 231 anal.								
2.0	2.0 cc.					.34	.34	.00	
	Supernatant 235 anal.								
2.0	2.0 cc.					.34	.34	.00	

* Pneumococcus I antibody solution prepared according to Felton.[4]

† The supernatant from this experiment, set up again with 0.5 cc. of PnI suspension, gave 0.01 mg. additional agglutinin N—a negative result within the limit of error of the method.

‡ Serum 231 agglutinated PnII"R" suspensions to a dilution of 1:160.
Serum 235 agglutinated PnII"R" suspensions to a dilution of 1:320.

notice. Quantitative evidence bearing on the mechanism of bacterial agglutination has also been obtained, and will be published later.

II. Quantitative relation between agglutinin and precipitin.

Although there is much in favor of the unitarian theory of antibodies, quantitative evidence for the identity of precipitins and agglutinins has hitherto been lacking.[5] This may now be supplied through the quantitative method for agglutinins outlined in the preceding note and the method for precipitins given in reference 3.

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[4] Felton, L. D., *J. Infectious Dis.*, 1928, **43**, 543.

[5] *Cf.* Zinsser, H., *J. Immunol.*, 1930, **18**, 483; also Felton, L. D., *J. Immunol.*, 1931, **21**, 341.

In order to minimize complications due to a multiplicity of antigens and antibodies, Type I anti-pneumococcus horse serum was absorbed with pneumococcus "C" substance and then with pneumococcus I "nucleoprotein" until no further precipitation occurred. A purified antibody solution was then prepared according to Felton.[4] As shown in Table II, the resulting solution contained 1.68 mg. of precipitin nitrogen per cc., as determined with the acetyl polysaccharide of Type I pneumococcus,[6] while the amount of agglutinin nitrogen per cc. was 1.86 mg. The discrepancy was found to be due to group specific antiprotein (or anti-C) which it had not been possible to remove by absorption of the serum, since the antibody solution yielded 0.22 mg. of agglutinin N per cc. with Pneumococcus II R suspension; an amount identical with that taken out by Pneumococcus I S suspension from the supernatants of the precipitin analyses. If this interpretation be accepted, the amount of anticarbohydrate agglutinin N was 1.86—0.22, or 1.64 mg. per cc.; in excellent agreement with the amount of anticarbohydrate N found by the precipitin method.

TABLE II.

Removal of Agglutinin or Precipitin from Pneumococcus I Antibody Solution B75.

Vol. antibody solution	Agglutinin N removed by Pn I suspension	Precipitin N found in supernatant	Total antibody N	Total antibody N per cc.	Precipitin N removed	Agglutinin N found in supernatant with Pn I suspension	Total antibody N	Total antibody N per cc.
cc.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0.5	.93	0	.93	1.86				
1.0					1.68	.22	1.90	1.90
0.5	.64	.27	.91	1.82				
1.0	.73	1.02	1.75	1.75	1.35	.50	1.85	1.85

It will be noticed that not only does the total agglutinin N correspond within a few hundredths of a mg. per cc. with total precipitin + agglutinin in the supernatant, but that partial agglutinin + residual precipitin, or partial precipitin + residual agglutinin determinations add to approximately the same total, regardless of the order in which the partial quantitative agglutinin or precipitin determinations are carried out. That the difference in the last instance in the table is as great as 0.1 mg. is undoubtedly due to antiprotein (or anti-C) remaining in the final supernatant, since only a little over one-third of the total antibody was removed as agglutinin.

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[6] Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

7236 C

Glutathione and Methylene Blue in Carbon Monoxide Poisoning.

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The experiments of Brooks[1] based upon theory derived from the investigations of Warburg *et al.*,[2] Meyerhof,[3] Heymans and Heymans,[4] Barron,[5] and others, aroused interest in methylene blue as an antidote for carbon monoxide poisoning. Many case reports (Geiger,[6] Bell,[7] Nass,[8] Christoferson,[9] Brown[10]) and some experimental reports (Brooks,[11] Draize,[12] Haggard and Greenberg[13]) have increased the literature upon this subject. Even the contradictory conclusions of Haggard and Greenberg and the concise summary of Henderson[14] have not sufficed to alter the popular conception of methylene blue as an antidote for carbon monoxide poisoning.

In the following experiments, the effect of glutathione upon carbon monoxide poisoning was first investigated because of Brooks' results with a similar oxidation-reduction catalyst, methylene blue. However, the discouragingly negative results soon prompted an investigation of methylene blue.

White rats (100 to 200 gm.) were placed one at a time in a special chamber through which carbon monoxide (produced by the action of hot sulphuric acid upon formic acid, purified by washing with solutions of potassium hydroxide and with water, and made up to 2% by volume in air) was passed at a uniform rate of approximately one-half liter per minute. The animals were removed from the chamber 30 seconds after the disappearance of the righting reflex and immediately injected intraperitoneally with warmed solution. Three solutions were used: (1) Methylene blue: 0.406 gm. of methylene blue U.S.P. Medicinal and 5.24 gm. of dextrose per 100 cc. of solution. (2) Glutathione: 0.60 gm. of glutathione (Eastman Kodak) and 4.00 gm. of dextrose per 100 cc. of solution. (3) Dextrose: 5.42 gm. of dextrose per 100 cc. of solution.

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[1] Brooks, M. M., *Am. J. Physiol.*, 1932, **102**, 145.

[2] Warburg, O., *Z. physiol. Chem.*, 1910, **66**, 305; *Biochem. Z.*, 1923, **162**, 518; 1926, **177**, 471; 1930, **227**, 245.

[3] Meyerhof, O., *Pflüger's Arch.*, 1912, **149**, 250.

[4] Heymans, J. F., and Heymans, C., *Arch. intern. Pharmacodynamie*, 1922, **26**, 443.

[5] Barron, E. S. G., *J. Biol. Chem.*, 1929, **81**, 445.

[6] Geiger, J. C., *J. Am. Med. Assn.*, 1933, **100**, 1103.

[7] Bell, M. A., *J. Am. Med. Assn.*, 1933, **100**, 1402.

[8] Nass, J., *J. Am. Med. Assn.*, 1933, **100**, 1862.

[9] Christoferson, A. W., *J. Am. Med. Assn.*, 1933, **100**, 2008.

[10] Brown, T. C., *Colorado Med.*, 1933, **30**, 302.

[11] Brooks, M. M., *Am. J. Physiol.*, 1933, **104**, 139.

[12] Draize, J. H., *Science*, 1933, **78**, 145.

[13] Haggard, H. W., and Greenberg, L. A., *J. Am. Med. Assn.*, 1933, **100**, 2001.

[14] Henderson, Y., *Science*, 1933, **78**, 408.

These solutions were freshly prepared before each run. The amount of each solution injected was 1 cc. per 100 gm. of body weight. The time of recovery was taken as the interval between the removal from the chamber and the return of the righting reflex. The time interval between the removal from the chamber and the return of the animal's ability to walk was also noted in an attempt to verify the results of Brooks,[1] though, as Haggard pointed out, this is not a reliable criterion for recovery.

RESULTS

Substance Injected	No. of Animals	Aver. time in chamber (min.)	Aver. time for recovery of righting reflex (min.)	Aver. time for recovery of ability to walk (min.)	Deaths within 5 min. after injection. Not included in preceding aver.
Dextrose	33	4.05 (100%)*	4.97 (100%)	7.85 (100%)	0
Methylene Blue	35	4.10 (101%)	4.67 (94%)	8.30 (106%)	4
Glutathione	15	4.25 (106%)	4.88 (98%)	8.16 (104%)	2

* The average times for dextrose-injected rats were taken as 100% and the other percentages were made on this basis.

It was noted that nearly all of the rats passed through an excitatory stage before collapsing.

The above results show that neither glutathione nor methylene blue injections have an appreciable accelerating effect upon the rate of recovery of the righting reflex in white rats poisoned with carbon monoxide. That such injections may prove to be fatal instead of beneficial was also shown.

Summary. 1. Glutathione injections did not appreciably accelerate recovery of white rats poisoned with carbon monoxide. 2. Methylene blue injections did not appreciably accelerate recovery of white rats poisoned with carbon monoxide. 3. Methylene blue injections appeared to be detrimental rather than beneficial to white rats suffering from carbon monoxide poisoning.

7237 P

A Thyreotropic Hormone from Pituitary Extracts.*

BORIS KRICHESKY. (Introduced by Bennett M. Allen.)

From the Department of Biological Sciences, University of California at Los Angeles.

The anterior pituitary substance responsible for thyroid hypertrophy and hyperplasia has been attributed to various anterior lobe hormones or to a separate thyreotropic hormone.[1] By heating a pituitary extract (Phyone), the thyreotropic substance has been separated from the contained gonad and growth stimulating substances.†

To determine the effect of heat on the activity of the growth hormone, young mice, 18 to 23 days of age, were used. 172 animals were divided into 5 groups: Group I injected subcutaneously with phyone; group II injected with phyone heated 60°C. 15 minutes; group III injected with phyone heated 80°C. 15 minutes; group IV injected with phyone heated to boiling 15 minutes. Group V was used as controls. All animals were injected with 0.1 cc. extract daily for 5 days. The average increase in body weight in 6 days was as follows: group I, 56%; group II, 55%; group III, 37%; group IV, 34%; controls, 35%. These data indicate that the growth hormone contained is not thermostable and is inactivated at some temperature between 60° and 80°C.

To determine the effect of heat upon the activity of the gonad stimulating hormone, female rabbits, 11 to 12 weeks of age, were used. These were divided into 3 groups of 7 each: group I, injected with 0.5 cc. of phyone, twice daily for 5 days; group II, with

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* Aided by a grant from the Board of Research, University of California, under the supervision of Dr. B. M. Allen.

[1] Riddle, O., Bates, R. W., Dykshorn, S. W., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 794. Anderson, E. M., and Collip, J. B., *Ibid.*, 1933, **30**, 680. Crew, F. A. E., and Wiesner, B. P., *Brit. J. Med.*, 1930, **1**, 777.

† Dr. David Klein, Wilson Laboratories, kindly supplied us with the Phyone used in this work.

1 cc. of phyone heated to boiling 15 minutes, twice daily for 5 days; group III, served as controls.

In group I all animals had hypertrophied uteri, and 6 showed from 2 to 4 corpora hemorrhagica in each ovary. No corpora hemorrhagica were present in group II, and in 6 animals the uteri were small and characteristically infantile, while the uterus of one animal, though small, showed a condition of hyperaemia. It is evident from these experiments that the gonad-stimulating hormone is inactivated by subjecting the extract to boiling temperatures for 15 minutes.

That the thyreotropic principle in phyone is still active even though the extract be heated to boiling, was determined by injection of this material into tadpoles. Injections of 0.1 cc. daily for 5 days into *R. catesbiana* larvae produces hypertrophy and hyperplasia of the thyroid gland. These glands show an emptying of the follicles of their contained colloid and a change in the follicular epithelium from the flattened to the columnar condition, indicating active secretion.

Heat treatment decreases slightly the effectiveness of the thyreotropic substance. Phyone, and phyone heated to boiling for 15 minutes, were made up to dilutions of 1:3, 1:6, 1:12, 1:24, 1:48, and 1:96. Each sample was injected into 6 tadpoles in doses of 0.1 cc. daily for 5 days. Hypertrophy and hyperplasia of the thyroid occurred in all animals receiving phyone up to and including dilutions of 1:48. Thyroids of animals receiving heated phyone showed similar hypertrophy and hyperplasia in dilutions up to and including 1:24, indicating a slight reduction in the activity of the thyreotropic substance.

Since the thyreotropic factor has been shown to be thermostable, it can be separated from the growth and gonad-stimulating substances by heat. These findings support the view that pituitary control of the thyroid is due to neither of these 2 hormones, but more probably to a separate and distinct thyreotropic hormone.

7238 P

Gastric Mucin as a Culture Medium for Intestinal Protozoa.

HERBERT L. RATCLIFFE. (Introduced by Balduin Lucké.)

From the Laboratory of Comparative Pathology, Philadelphia Zoological Society, and the Department of Pathology Medical School, University of Pennsylvania.

Mucin is a natural constituent of the intestinal contents; hence the assumption that it might be used as a culture medium for intestinal protozoa. So far as can be determined mucin has not been employed heretofore for this purpose. Samples of gastric mucin prepared by both Armour and Company and the Wilson Laboratories were used. These are dry powders, readily soluble in aqueous media and solutions may be sterilized in the autoclave.

A medium, composed of liver infusion agar slants[1] covered with a solution of 0.3% mucin in 0.7% aqueous NaCl with a 2 mm. loop of sterile rice starch to each tube, was used to isolate *Endamoeba histolytica* from one case of acute amoebiasis, from one human carrier and from 3 *Macaca irus* that were carriers. The amoeba grew in moderate numbers but cysts were not formed. Subcultures were made at 2 or 3 day intervals.

Trichomonas sp. from *Pan niger, Cebus fatuellus, Macaca irus* and *Tapirus bairdii* grew in abundance in a medium of 0.3% mucin in 0.7% NaCl solution. Subcultures were made at 3 to 4 day intervals. *Trichomonas sp.* and *Eutrichomastix sp.* from various reptiles have grown in abundance in 0.3% mucin in 0.5% aqueous NaCl solution, with subcultures every 7 days. A 2 mm. loop of rice starch to each tube seemed to increase the rate of growth of the flagellates from both mammals and reptiles. This medium, 0.3% mucin in 0.5% aqueous NaCl solution with rice starch, was used also to isolate 4 strains of *Endamoeba sp.* from *Natrix sipedon* and *N. taxispilota.* Growth and cyst formation was abundant. Subcultures were made every 7 days. *Balantidium sp.* from the intestine of *Testuda galapogoensis* has been cultured on this medium also, transfers being made every 7 days. The ciliates grew very well but did not encyst.

Blastocystis, although usually present in fecal material from which these protozoa were isolated, grew poorly in the media containing mucin, and often disappeared after a few subcultures.

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[1] Cleveland, L. R., and Collier, J., *Am. J. Hyg.*, 1930, **12**, 606.

7239 C

Studies on the Crystalline Lens. Fate of Glutathione in Parathyroid Cataract.

EVERETTE I. EVANS. (Introduced by A. B. Luckhardt.)

From the Department of Physiology, University of Chicago.

It is well established that in senile cataract two of the principal biochemical changes are (1) a great increase of calcium and (2) an apparent total loss of glutathione.[1] Evans and Kern[2] demonstrated that in experimentally produced parathyroid cataract (by extirpation of the parathyroid glands), the calcium content of these cataractous lenses increased to approximately 25 times the amount present in normal dog lens.

It is probable that glutathione plays a definite rôle in the internal oxidation-reduction and enzymic reactions of the lens (initially suggested by Goldschmidt[3]). We were therefore naturally interested to learn the fate of the glutathione of the crystalline lens after removal of the parathyroids. Preliminary study indicated that the normal dog lens contains approximately 0.385% reduced glutathione, and that any figure considerably below this might be considered as indicating a loss of glutathione from the lens. The opportunity to study the fate of glutathione in parathyroid cataract was afforded by the early development of bilateral lenticular opacities in 5 young dogs (2 mos. old) that had been thyroparathyroidectomized in connection with another study. A complete description of these lenticular changes has been made elsewhere.[4]

At the time the dogs were sacrificed because of severe distemper, they had been without parathyroids for 57-152 days. The earliest signs of lenticular degeneration were evident at about the 25th day. The dogs were kept on a sour milk-bread-liver diet; tetany was not observed.[5] The dogs were sacrificed by the intravenous injection of small amounts of sodium cyanide. Immediately after death the eyes were excised, the crystalline lenses removed and weighed on a watch glass.

Glutathione determination: Reduced glutathione was determined by the Okuda Method as modified by Hess[6] for blood. The 2 lenses were ground with 10 cc. 10% sulphosalicylic acid and filtered by suction through hard paper. The protein residue was reground twice with 5 cc. portions of the same acid and the cake washed with two 2 cc. portions of the acid. The combined filtrates were made up to 30 cc. and an aliquot taken for immediate titration with M/1200 KIO_3 under conditions as specified by Okuda.[7] For determination of oxidized glutathione, an aliquot was reduced with zinc and HCl. In *2* cases an aliquot was hydrolyzed with 20% HCl for 6 hours and the liberated cystine determined by the Sullivan Method.[8] Also, in 3 cases, a Sullivan test for free cystine or cysteine was made directly on an aliquot of the sulphosalicylic acid extract. These data and other unpublished data (in collaboration with Drs. Sullivan and Hess) on glutathione in the crystalline lens indicate that the Okuda Method, when used on the sulphosalicylic acid extracts of the crystalline lens, titrates only glutathione of the reducing substances present.

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[1] See discussion in Duke-Elder's Textbook of Ophthalmology, London, Henry Kimpton, 1932, p. 481.

[2] Evans, E. I., and Kern, R., *Am. J. Ophth.*, 1931, **14**, 1029.

[3] Goldschmidt, M., *Arch. f. Ophth.*, 1924, **113**, 160.

[4] Evans, E. I., *Am. J. Ophth.*, in press.

[5] Evans, E. I., *Am. J. Physiol.*, 1933, **105**, 32.

TABLE I.
Glutathione in the cataractous lenses.

Dog No.	Days alive after operation	Free cysteine or cystine	Reduced Glutathione %	Oxidized Glutathione %	Total Glutathione %
1	57	....	0.115	....	0.115
2	82	....	0.132	0.021	0.153
3	142	None	0.110	0.009	0.119
4	138	”	0.184	0.014	0.198
5	152	”	0.121	0.015	0.137
6-15*	....	”	0.385	0.007	0.392
16-23†	....	”	0.391	0.002	0.393

* 10 normal adult dogs.
† 8 normal young dogs.

Summary. The data shown in Table I indicate that before any appreciable calcium deposition takes place in the developing "parathyroid cataract" there is a serious loss of glutathione from the lens, amounting in the cases described here, from 53 to 72% of the amount present normally. Also, the negative Sullivan reaction for the lens filtrates indicates that free cystine is not the end-product of the destroyed glutathione.

If glutathione is as necessary for certain enzymic reactions and internal autoxidations as it is held by many to be, it is very probable that its removal or destruction must seriously impair the metabolism of the lens. Just how glutathione is destroyed after removal of the

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[6] Hess, W. C., *J. Wash. Acad. Sci.*, 1929, **19**, 419.

[7] Okuda, Y., *J. Dept. Agri., Kyushu Imp. Univ.*, 1929, **2**, 133.

[8] Sullivan, M. X., and Hess, W. C., *U. S. P. H. S., Public Health Reports*, Suppl. No. 86, 1930.

parathyroids is not clear but it may not be impertinent to suggest that it might be removed with glutathione from the other tissues, to detoxify certain injurious metabolic products that may arise after parathyroidectomy. Each of the 3 amino acids, glycine, glutamic acid, and cysteine are important detoxifying agents.[9]

These data on glutathione in parathyroid cataract suggest further the possible rôle of parathyroid deficiency (even in the absence of any tetany) in the pathogenesis of senile cataract.

7240 P

A Simple Method for Production of Vitamin D Milk of Known and Controllable Potency.

DAVID H. SHELLING AND H. C. TIDWELL.

From the Harriet Lane Home, Johns Hopkins Hospital, and the Department of Pediatrics, Johns Hopkins University.

Since the introduction of irradiated ergosterol and irradiated foods for the prevention and treatment of rickets, several methods have been employed in order to impart anti-rachitic properties to milk. Among these may be mentioned: (1) the irradiation of milk in both the powdered and liquid forms; (2) irradiation of the cow; and, (3) feeding to the cow substances containing large amounts of vitamin D, as for example cod liver oil or irradiated yeast. The irradiation of the cow as a means of imparting vitamin D to the milk is obviously most impractical from the commercial point of view, as is also the feeding of large amounts of cod liver oil. The irradiation of the milk itself has the practical disadvantage of being expensive since it involves the installation, maintenance and operation of expensive apparatus in the farm or dairy where it is employed. The direct irradiation of the milk and the indirect method of feeding irradiated yeast to the cow both have the disadvantage that they are not absolutely reliable methods of holding the vitamin D content at fixed concentration, so that repeated testing of the milk at regular intervals becomes necessary in order to be sure that the vitamin D content of the milk has not fallen below the standard.

In view of these difficulties it occurred to us that the method for adding fats to milk, which has been employed successfully by Holt, Tidwell and their associates, in our laboratories, for the past 4 years, might also be more practical for fortifying milk with vitamin D. Our procedure consists simply of homogenizing, in water, a concentrated oily solution of viosterol of known potency, with the aid of an emulsifying agent. We have tried several emulsifying substances, which are quite feasible as such, but in order to produce an emulsion which will be stable for months or years under ordinary environmental conditions, we have found lecithin to meet this requirement. This stable emulsion appears white as milk, is miscible with it, and has no objectionable odor or taste. It may be kept in the ice box, under ordinary conditions; it could be aerated with nitrogen, sealed in vials, and shipped for distribution. Since each cc. of the emulsion is of known anti-rachitic potency, a given amount of the emulsion may be thus added to a measured amount of milk so as to impart to it a desired number of *international* or *rat units* per quart. The emulsion may be added to the milk before pasteurization, as this procedure does not impair the vitamin D potency.*

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[9] **Brand, E., and Harris, M. M., *Science*, 1933, 77, 589.**

The following figures exemplify the amounts of the emulsion necessary to impart to each quart of milk an amount of vitamin D equal to 5 or 10 drops of viosterol 250-D.† If the original viosterol concentrate contains 25,000 D per cc. then a 5.0% emulsion will be 1250 D per cc., or 5 times the potency of the commercial viosterol—250 D. Since oily solutions contain about 45 drops per each cc., it is thus necessary to add but one cc. of the emulsion to 45 or 22.5 quarts of milk, depending whether 5 or 10 drops of viosterol is desired in each quart. The potency of the emulsion could be increased by using a more concentrated viosterol preparation or by increasing the percentage of viosterol concentrate in the emulsion.

We have tested the emulsion after several months standing and found it to be as potent as the original viosterol and not to have lost its potency after 15 minutes heating at 100° C. Further work on animals as well as clinical tests on a group of colored babies is now in progress. The results will be published in detail at a future date.

We believe that this method of fortifying milk with vitamin D is the most practical, since it is the simplest and is controllable and offers a universal means of supplying vitamin D to those who are in need of it and would not otherwise receive it. It ought to be much less expensive than the other methods. Its cost will depend on the price of viosterol as determined by the Wisconsin Foundation.

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* In dairies equipped with homogenizers the concentrated viosterol may be added to a small amount of fresh milk, homogenized, and added to a calculated quantity of milk before it is pasteurized.

† 37 rat units = 100 international vitamin D units.

Missouri Section.

Washington University Medical School, January 10, 1934.

7241 P

Observations upon Conduct of a Thalamic Dog.[*] Hearing and Vision in Decorticated Animals.[†]

E. A. CULLER AND FRED A. METTLER. (Introduced by Alrick B. Hertzman.)

From the Laboratory of Physiological Psychology, University of Illinois, and Department of Physiology, St. Louis University School of Medicine.

Some time ago one of us observed conditioned visceral responses in rabbits with extensive pallial destruction.[1] Acting upon this evidence and with the knowledge that the cortex was considered the usual if not only locus of the conditioning process by Pavlov[2] on the basis of work by Zeliony[3] we attempted to secure a decorticated animal higher in the evolutionary scale, and here describe the behavior of a decorticated dog with intact thalamus. Almost a hundred animals were utilized in this work which extended over a period of nearly 3 years.

A detailed record of the behavior of the animal was kept beginning with the time of operation (done in 2 stages). The day after operation the animal was able to stand rigidly on its feet when put in that position (extensor hypertonia) but slowly sank to the floor under its own weight without moving. Rigidity of the extremities with clasp-knife reflexes (Creed[4]) was observed until its death. In 3 days, the animal was able to move with difficulty (which is exactly the time Goltz gives), in 5, it would stand for hours without motion but when in motion moved in a straight line. For 8 days no reaction to pistol shots nor a flash lamp beam was discerned, but on the ninth day a pupillary reflex was present and on the tenth the animal reacted to sound (same day Rothmann's dog registered). It grew to use its muzzle sensibility for purposes of directing its locomotion (Rothmann[5]) keeping its head low to the floor. The animal reacted to olfactory sensations on the ninth day but never ate or drank spontaneously. On the tenth day circus movements appeared and grew stronger with occasional convulsions and somatic weakening until killed on the 19th day. During its life it showed marked resistance to impressed movements, was unable to respond promptly to stimulation nor could it cease its own activity until this wore off gradually. (Efforts were made to observe sympathetic excitation and phenomena which might be considered sham rage.)

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[*] Financed by the Research Bureau of the American Otological Society. Certain financial assistance by the Sarah Manning Sage Fund (Cornell University Medical College, Ithaca, New York) and the School of Medicine, St. Louis University is acknowledged.

[†] Communication No. 3 from the Alpha Research Laboratory, University of Illinois.

[1] Unpublished observations by Fred A. Mettler.

[2] Pavlov, I., Conditioned Reflexes, Oxford University Press, 1927.

[3] Zeliony, T. N., *Trans. Soc. Russian Physicians of St. Petersburg*, 1911-12, **50**, 147.

[4] Creed, Denny-Brown, Eccles, Liddell and Sherrington. Reflex Activity of the Spinal Cord. Oxford, Claredon Press. 1932.

Conditioned training was begun on the 11th day according to a usual technique (Girden and Culler[6]) and before death, the animal gave definite evidence of conditioning to sound. Light was also used, but more complete records were kept on sound. Before conditioned training was begun the animal reacted to a loud bell merely by moving the ears. This sound was used as the conditioned stimulus in association with a shock to the forepaw. On the last day tested the animal evinced motor reactions which had developed during the conditioned training 20 times out of 25 trials with 2 doubtful and 3 negative reactions.

Postmortem examination disclosed enormous thickening of the dura, intracranially, especially over the basis cranii. There was a thick fibrous pad over the remnants of the brain which pad extended by trabeculae into the brain substance and was continuous with a cyst situated in the medullary center of the left hemisphere. This cyst extended from the situation of the presylvian sulcus to the situation of the posterior lateral gyrus. A tab of the frontal lobe on both sides was adherent to the piriform area but the connections were severed and it was later seen to be more or less acellular. A similar situation maintained with regard to a tab of cortex in the depth of the left Sylvian sulcus. The head of the caudate nucleus was invaded by fibrous tissue on the left side and the third ventricle had also been invaded. A complete microscopic study of the brain has been made by the Marchi method (Mettler[7]).

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[5] Rothmann, H., *Z. f. d. ges. Neur. u. Psychiat.*, 1923, **87**, 247.

[6] Girden and Culler, *Am. J. Roent. and Rad. Ther.*, 1933, **30**, 215.

There can be no doubt from this and accompanying experiments that deafness and blindness produced by cortical removal are not identical with destruction of the peripheral apparatus. Although there is an initial period of complete deafness, this passes away (Rothmann's[5] animal gave evidence of hearing on the same day after operation as ours) very suddenly. Although the animal above could smell (tests for taste were unsatisfactory) it would not eat spontaneously. The reason for this is not immediately apparent but sometimes occurs in dogs when only one frontal lobe is injured. (Further work on this point is in preparation.) Evidence is available to show that the behavior of this animal was modifiable by training. Recently Zeliony in association with Poltygrew[8, 9] has reversed his earlier view. Rothmann[5] states too that his animal was able to learn to do simple tricks. Herrick has long called attention to modifiability of behavior at thalamic levels and vicarious functioning of cortical tissue has been for some time open to serious question. The conditioned reaction in this animal was in the form of a pattern rather different from the usual and suggested nervous mediation by an intrinsic spinal mechanism (Creed et Alius[4]) rather than the corticospinal tract. Zeliony himself wrote "We have to keep in mind that our dog was abnormal and therefore it would be possible that some deviation from the normal response would be expected if conditioned reflexes were possible." The specific qualities of the "motorium" were absent in this animal, *i. e.,* it was unable to activate a movement suddenly or to stop its own activity suddenly. There appeared to be autonomic overactivity in this and similar animals as would be expected from the results of Fulton and Ingraham.[10] The above results must be interpreted to mean that decorticated animals can distinguish sound and may be conditioned to it. As nearly as the authors are aware, this is the first presentation of positive evidence of conditioning in a thalamic animal with postmortem examination and microscopic study.

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[7] Mettler, Fred A., *Stain Tech.*, 1932, **7**, 95.

[8] Poltygrew and Zeliony, *Am. J. Phys.*, 1929, **90**, 475.

[9] Poltygrew and Zeliony, *Z. J. Biol.*, 1930, **90**, 157.

[10] Fulton and Ingraham, Proc. Phys. Soc., in *J. Physiol.*, 1929, **67**, 27.

7242 C

Intrapleural Pressure in Orthopnea.

MYRON PRINZMETAL AND WILLIAM B. KOUNTZ.

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Among the various theories concerning the etiology of orthopnea much support has been given to the idea that pulmonary ventilation is facilitated by the upright position. It has been shown that the vital capacity is generally higher,[1] that the total volume of the lung is greater,[2] that the mid capacity is greater,[3] that the accessory muscles of inspiration can be used to better advantage,[4] and that the diaphragm is lower[5] than in the recumbent position.

Aron[6] found in one normal individual that the intrapleural pressure was less negative when he was recumbent. There has, however, been no systematic study of the intrapleural pressure in pathological states leading to orthopnea. For this reason, we have taken intrapleural pressures in both the recumbent and upright positions in 13 individuals, 6 of whom were without orthopnea. Of 7 patients with orthopnea, 3 had cardiac decompensation, 2 were asthmatic, one had extreme pulmonary emphysema with bronchitis and one had marked ascites due to cirrhosis of the liver.

In all cases we found the intrapleural pressure to be less negative in the recumbent than in the upright position. This shift was more marked in the patients with orthopnea than in the control group. Occasionally the intrapleural pressure rose above atmospheric pressure in the recumbent position.

These observations support the mechanical theory of orthopnea. A less negative intrapleural pressure will limit the pulmonary ventilation and, according to the work of Starling,[7] interfere also with pulmonary circulation. By such a mechanism a vicious circle may be established, further congestion leading to greater interference with the ventilation of the lungs. In those cases in which a positive intrapleural pressure occurred during recumbency, direct pressure on the heart might interfere with its action.

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[1] Christie, C. D., and Beams, A. J., *Arch. Int. Med.*, 1923, **31**, 85.

[2] Hurtado, Alberto, and Fray, Walter W., *J. Clin. Invest.*, 1933, **12**, 825.

[3] Bohr, C., *Arch. f. klin. Med.*, 1907, **88**, 385.

[4] Hewlett, A. W., *Monographic Medicine,* **1**, 375.

[5] Hofbauer, L., *Z. f. klin. Med.*, 1907, **61**, 389; 1914, **19**, 128.

[6] Aron, E., *Virchow's Arch. f. Path. Anat.*, 126, 517, 189, quoted from Emerson, *Bull. Johns Hopkins Hosp. Reports*, 1903, **2**, 193.

[7] Wright, S., Applied Physiology, 3rd Edition, 1929, 299.

TABLE I.

				Intrapleural Pressure				
				Upright		Recumbent		
Sex	Age	Diagnosis	Orthopnea	Insp.	Exp.	Insp.	Exp.	Aver. Change
F	49	Asthma and Emphysema	Moderate	— 8 cm. H_2O	0 cm. H_2O	—10 cm. H_2O	+ 6 cm. H_2O	+ 4 cm. H_2O
M	26	Asthma	Marked	—16 ”	—12 ”	— 8 ”	— 4 ”	+10 ”
					one minute later	— 6 ”	— 2 ”	
M	43	Emphysema	”	— 4 ”	— 1 ”	— 2 ”	+ 1 ”	+ 2 ”
M	65	Cardiac	Moderate	— 8 ”	— 6 ”	— 3 ”	— 2 ”	+ 4.5 ”
M	60	”	Marked	— 7 ”	— 4 ”	— 2 ”	0 ”	+ 4.5 ”
M	50	Cardiac Marked Ascites	Moderate	— 6 ”	+ 4 ”	— 4 ”	+ 6 ”	+ 2 ”
			Aver. for group—4.5 cm. H_2O more positive in recumbent position.					
M	26	Case 2 (without asthma)	No	— 5 ”	— 2 ”	— 3 ”	— 2 ”	+ 1 ”
M	33	Tb with Pneumothorax	”	— 8 ”	— 2.5 ”	— 3.5 ”	— 1.5 ”	+ 2.75 ”
M	25	” ” ”	”	— 5 ”	— 1 ”	— 3.5 ”	+ 1 ”	+ 1.75 ”
M	27	” ” ”	”	—14 ”	— 4 ”	— 8 ”	— 1 ”	+ 4.5 ”
M	30	” ” ”	”	— 7 ”	— 2 ”	— 6 ”	0 ”	+ 1.5 ”
M	35	” ” ”	”	—14 ”	— 4 ”	— 8 ”	0 ”	+ 5 ”
M	30	” ” ”	”	— 4 ”	0 ”	— 2 ”	+ 3 ”	+ 2.5 ”

Aver. for group— +2.7 cm. H_2O more positive in recumbent position.
Total aver.— +3.5 cm. H_2O more positive in recumbent position.

7243 P

Influence of Inversion of the Dog on the Electrocardiogram.

W. B. KOUNTZ AND M. PRINZMETAL.

From the Department of Medicine, Washington University, and the Barnes Hospital, St. Louis.

From the classical observations of Lewis[1, 2] in dogs it has usually been inferred that the initial ventricular deflections of extrasystoles arising from the right ventricle are down in lead I and up in lead III, while those arising from the left ventricle are up in lead I and down in lead III. These observations were generally accepted in the interpretation of the human electrocardiogram until it was shown by Barker[3] that on stimulation of the human heart extrasystoles were produced which were opposite in character to those obtained in the dog.

We found in dogs with an experimentally produced chronic pericarditis that the initial deflection of ventricular extrasystoles may be reversed from the usual type described by Lewis.[1, 2] In these experiments it was noted that the heart did not fall away from the anterior chest as is always the case in the normal animal, an observation which suggested that the change might be due to the fact that the heart was held in its normal anterior position when the chest was opened.

To test this hypothesis the following experiment was performed on 6 normal dogs. An animal was anesthetized and securely fastened on its back to a board. The chest and pericardium were opened in the usual manner. Electrocardiographic tracings were made and at the same time 7 points on the ventricle designated by Barker[3] were stimulated. The board was then turned over so that the animal was suspended with its anterior surface to the floor. This allowed the heart to fall against the anterior chest wall, was always accompanied by slight rotation of the heart to the right, and represented the more nearly normal position of the heart in the dog. Extrasystoles were again obtained by stimulating the identical 7 points. The animal was then placed on its back and the right bundle was

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[1] Lewis, T., The Mechanism and Graphic Registration of the Heart Beat, 1925, London.

[2] Lewis and Rothschild, *Phil. Trans. Royal Soc.* (B), 1915, **206**, 181.

[3] Barker, P. S., McCleod, A. G., and Alexander, J., *Am. Heart J.*, 1929, **5**, 720.

cut. Electrocardiograms were taken in this position and also after inversion of the animal.

When the dog was on its back with the heart against the spine we usually confirmed Lewis'[1, 2] observations. By inverting the dog, which allowed the heart to return to its more nearly normal position, complete reversal of the usual type of extrasystoles and bundle branch block was obtained.

These results suggest that the different findings obtained by Lewis in the dog and by Barker in the human can be explained by the position of the heart in the thorax. Due to the deep anterior posterior diameter of the chest in the dog the heart falls from its normal anterior position when the pericardium is opened, a change which is not mechanically possible in the relatively shallow mediastinum of man.

7244 C

Effects of Ergotamine, Adrenalin, Atropine, and Calcium on Thyroid Gland of the Guinea Pig.*

A. ARNOLD KIPPEN. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis.

Because of the relations of the autonomic nervous system to the thyroid gland, we thought it of interest to determine the effects of certain drugs which are known or believed to affect the autonomic nerves in a specific manner.

We tested the effect of these drugs in two ways: (1) We either injected solutions of these drugs subcutaneously or intraperitoneally, or administered them orally, and then examined the thyroid gland microscopically, noting the various characteristic features considered in the previous investigations from this laboratory; in particular making approximate counts of mitoses in various glands. (2) We determined the effect of these drugs on the hypertrophy and hyperplasia which is produced in the thyroid by injections of extracts of anterior pituitary gland.

Male guinea pigs, the initial weights of which ranged between 200 and 300 gm., were used for our experiments.

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* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

Experiments with Ergotamine Tartrate (Gynergen). To 8 guinea pigs ergotamine tartrate was given daily for 6 to 9 days in doses varying between 0.5 mg. and 6.0 mg. The drug was administered partly by mouth, and partly by subcutaneous injections. No toxic effects were noted. With the smaller doses there was a gain in weight, but with the larger doses the weight either remained stationary or showed a slight loss. Microscopically the thyroids were those of normal guinea pigs, as far as amount and character of colloid, relative number of mitoses and lack of hypertrophy are concerned.

In 9 guinea pigs, ergotamine and anterior pituitary extract were administered at the same time, 0.1 cc. or 0.3 cc. anterior pituitary extract being injected daily intraperitoneally, for periods of 6 or 9 days, and simultaneously 5.0 mg. ergotamine being given orally and 0.5 mg. injected subcutaneously. Controls were injected with anterior pituitary extract alone.

No definite differences were found between the thyroids of the animals receiving ergotamine plus anterior pituitary extract and the controls.

Experiments with Adrenalin. In 4 guinea pigs, adrenalin hydrochloride (1:1000) was given intraperitoneally in doses of 0.1 cc. or 0.3 cc. daily for 5 to 9 days. With the 0.1 cc. dose there was a gain in weight, but with the 0.3 cc. doses there was a definite loss in weight. In all cases the gross appearance and the microscopic picture of the thyroids were essentially normal.

In 3 guinea pigs, 0.3 cc. adrenalin hydrochloride (1:1000) and 0.3 cc. anterior pituitary extract were simultaneously injected intraperitoneally daily for 6 and 9 days. There was no marked difference between the activity of the thyroids of animals receiving adrenalin plus anterior pituitary extract and controls injected with anterior pituitary extract alone.

The fatal intraperitoneal dose of adrenalin hydrochloride (1:1000) for a guinea pig, weighing approximately 200 gm., varies between 0.2 cc. and 0.5 cc. Almost immediate death occurred in some of the injected animals, preceded by jumping movements, ataxia, and in several cases there was bleeding from the nose. In several other cases the guinea pigs died after a few daily injections of 0.3 cc. These animals showed extreme weakness and a marked loss of weight.

Experiments with Atropine Sulphate. Atropine sulphate was injected into 9 guinea pigs, in most cases intraperitoneally, but in 2 cases subcutaneously, in daily doses varying between 0.3 mg. and

40.0 mg., for periods of 7 to 10 days. The thyroid of one of the animals which received larger doses showed a marked hypertrophy, and another one a slight hypertrophy. In all other cases the thyroids were normal.

In the guinea pigs in which 36 to 40 mg. atropine sulphate were given daily intraperitoneally, the palpebral fissures became slit-like within a short time and the eyes watered considerably. For a guinea pig weighing about 200 gm., approximately 40.0 mg. atropine sulphate injected intraperitoneally represents a toxic dose.

Ten guinea pigs received daily subcutaneous injections of 30 or 40 mg. atropine and simultaneous intraperitoneal injections of 0.3 cc. to 1.0 cc. of anterior pituitary extract. Atropine did not noticeably influence the hypertrophic changes produced by the extract.

Experiments with Calcium Gluconate. Calcium gluconate was injected intraperitoneally into 4 guinea pigs in doses of 0.2 gm. daily for 7 and 11 days. Grossly and microscopically thyroids as well as parathyroids appeared normal.

Ten guinea pigs received simultaneous daily injections of 0.1 cc. or 0.3 cc. anterior pituitary extract, and 0.2 gm. calcium gluconate. In 2 cases the calcium gluconate was administered orally, in 2 cases subcutaneously, and in all other cases intraperitoneally.

The thyroids of the animals receiving calcium did not appreciably differ from the thyroids of controls injected with anterior pituitary extract alone.

We may therefore conclude that (1) ergotamine tartrate (Gynergen), adrenalin hydrochloride, atropine sulphate, and calcium gluconate, which are known or believed to act in a specific manner on the autonomic nervous system, have no appreciable effect on the thyroid gland of the male guinea pig with the doses used; and that (2) these substances when given in conjunction with extracts of anterior pituitary gland do not noticeably alter the effects of the latter on the thyroid gland of the male guinea pig.

Cleveland Section

Western Reserve University, January 12, 1934.

7245

Glutathione Content of Blood.

RUSSEL O. BOWMAN. (Introduced by V. C. Myers.)

From the Department of Biochemistry, School of Medicine, Western Reserve University.

The iodate titration method of Hess,[1] with slight modifications, has been applied to the determination of reduced glutathione in tungstic acid filtrates of blood in a series of almost 1000 cases. The method is accurate to 2 mg. per 100 cc., and the only known blood constituents to interfere are thioneine and possibly vitamin C. Thioneine is partly precipitated by tungstic acid, and could account maximally for only 2 mg. per 100 cc. The effect of vitamin C was not determined but should be insignificant.

Cell volumes by the hematocrit method were determined on most of the cases and reduced glutathione was expressed also on the basis of 100 cc. of cells.

Ninety-three normals gave a mean value of 27.5 mg. per 100 cc. of whole blood and 69.5 mg. per 100 cc. of cells. Variations were from 8 to 46 mg. per 100 cc. of whole blood.

Three hundred forty-two diabetic patients gave a mean value of 25.1 mg. per 100 cc. of whole blood and 66.1 mg. per 100 cc. of cells. The reduced glutathione did not vary consistently with insulin therapy, and did not vary with the level of blood sugar. In a smaller series of cases, reduced glutathione did not correlate with the saccharoids of the blood.

Eighty-eight nephritis cases showed a mean of 21.8 mg. per 100 cc. of whole blood and 68.4 mg. per 100 cc. of cells. Reduced glutathione did not accumulate in the blood when other nitrogen products were retained.

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[1] Hess, W. C., *J. Washington Acad. Sciences*, 1929, **19**, 419.

Series of determinations in cases of prostatitis, circulatory diseases, liver damage, fevers, tuberculosis, pneumonia, cancer and other pathological conditions, showed no direct correlation of reduced glutathione with the clinical diagnosis. In general, when the cell volume was low in anemic cases, the whole blood reduced glutathione was reduced below the normal mean, and the figure per 100 cc. of cells was high (80 to 90 mg.). The opposite was true for polycythemic cases.

No relation could be established for reduced glutathione with clinical condition, exercise, diet, age, sex or color. Reduced glutathione usually varied with the hemoglobin value, but not constantly.

Unexplained variations of reduced glutathione for the same individual, and for different individuals with similar pathological pictures, were found. These were not due to oxidized glutathione since the same variations were obtained using the Benedict-Gotschall[2] colorimetric method for total glutathione.

The results are mostly negative in character. They indicate that reduced glutathione is not a physiological constant, but tends to be such. Further work is necessary on the complex function of glutathione in the body, before the results can be interpreted.

7246 P

Magnitude of Urinary Iron Excretion in Healthy Men.

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Henriques and Roland[1] have found that the daily excretion of iron in the urine of both normal and pathological cases is very small, amounting to only 0.08 to 0.32 mg. These values are much lower than those reported by earlier workers. Lintzel,[2] however, has claimed that not more than 0.02 mg. of iron per liter can be found in normal urine. Likewise, other investigators[3] have found the urine of patients suffering from various diseases to be practically iron-free. Because of its bearing on problems relating to the metabolism of iron we have investigated anew the question of the magnitude of the urinary iron excretion.

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[2] Benedict, S. R., and Gotschall, G., *J. Biol. Chem.*, 1933, **99**, 729.

[1] Henriques, V., and Roland, H., *Biochem. Z.*, 1928, **201**, 479.

[2] Lintzel, W., *Z. f. Biol.*, 1929, **89**, 350.

[3] Lanyar, F., Lieb, H., and Verdino, A., *Z. f. physiol. Chem.*, 1933, **217**, 160.

The urine was obtained in clean bottles from male subjects who were pursuing their usual routine in the laboratory. In brief, the analytical procedure consisted of the following steps: A measured volume, usually 500 cc., of the fresh 24-hour urine specimen was evaporated to dryness in a silica dish, and then incinerated in an electric furnace at about 500°C. for 8 hours. The ash was dissolved in dilute HCl, brought to a volume of 50 cc., and aliquot portions were removed for the determination of iron by the thioglycolic acid method.[4]

The analytical technic was subjected to repeated tests and found to be adequate. All reagents were shown to be free from iron. Small quantities of iron added to urine were quantitatively recovered, proving that iron was not lost in the manipulations. Finally, a solution of pure substances was prepared in imitation of the main constituents of urine and this solution was evaporated, ashed, and analyzed for iron. None could be detected, proving that the iron found in the analyses was not due to contamination.

In 32 samples of clear urine from 3 healthy subjects the minimum and maximum values were 0.13 mg. and 0.42 mg. of iron per day. The average urinary excretion for each subject was 0.20, 0.24, and 0.30 mg. The individual variations from day to day were all within ±50% of the mean values. These fluctuations may be due to diet or other factors.

The nature of the urinary iron remains to be determined. Some of the iron of normal urine can be removed by filtration through a thick asbestos mat in a Gooch crucible. This indicates that part of the urinary iron is combined with, or present in, the formed elements of normal urine. Regardless of the nature of the urinary iron, it is evident that its determination should not be neglected in precise balance studies of the intakes and excretions of this element.

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[4] Hanzal, R. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 846.

7247 P

Metabolism of Iron and Copper in Anemic Rats.

FRANKLIN C. BING, ESTHER M. SAURWEIN AND VICTOR C. MYERS.

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Rats were made anemic by restriction to a diet of cow's milk. This milk was obtained directly in glass bottles from Guernsey cows and contained an average of 0.14 mg. of copper per liter. One group of 5 anemic rats was sacrificed. The stomachs and intestines were removed, and the carcasses were analyzed for iron and copper. Another group of 5 rats received daily supplements of 0.5 mg. of iron as pure ferric chloride. A third group of 6 rats received daily intraperitoneal injections of 0.5 mg. of iron, and a fourth group received 0.5 mg. of iron and 0.025 mg. of copper as copper sulfate each day *per os.*

Hemoglobin determinations were made at intervals on blood samples secured from the tails. In 17 days the animals receiving iron by mouth had increased their hemoglobin from an average concentration of 4.15 gm. to 7.23 gm. per 100 cc. of blood. The rats receiving iron intraperitoneally increased their hemoglobin from 4.03 gm. to 12.92 gm. in the same time, and the rats receiving iron and copper orally increased their hemoglobin from 3.90 gm. to 13.40 gm. per 100 cc. of blood. These data confirm conclusions reached in previous work from this laboratory,[1] namely, that supplements of iron bring about some hemoglobin production in anemic rats and that intraperitoneal injections of iron result in hemoglobin production at about the same rate and to the same extent as oral administrations of both iron and copper.

TABLE I.
Average Values of the Iron and Copper Contents of the Rats.

Group	No. Rats	Net Body Wt. gm.	Iron Content mg.	Copper Content mg.
1. Controls	5	58	1.13	0.051
2. Fe orally	5	68	2.12	0.061
3. Fe injected	6	88	6.78	0.104
4. Fe + Cu orally	5	87	3.63	0.115

The average values for the analyses of the carcasses of the rats for iron and copper are recorded in Table I. These figures show that the hemoglobin production in the rats receiving injections of iron is associated with an increase in the copper content of the body over that of control rats sacrificed at the beginning of the experiment. It has been calculated that the average retention of copper in this group of rats was 73% of the total intake in the form of the copper of the milk, while in the group receiving iron orally it was 17% and, in the group receiving both iron and copper, only 12% of the total intake during the experimental period.

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[1] Eveleth, M. W., Bing, F. C., and Myers, V. C., *J. Biol. Chem.*, 1933, **101**, 359.

7248

Carotenemia. Its Influence on the Validity of the Icteric Index.

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In a paper with Hess[1] one of us called attention to the occurrence of carotenemia. At the time it was pointed out that this might in certain cases cause confusion with icterus. Later it was observed that the carotenemia might be very intense in diabetic patients on the high vegetable diet of the time.

It is obvious that carotene must exert some influence on the icteric index of the blood, a fact which has been appreciated by most of the workers in this field. Fiessinger, Walker and Thierry[2] from their work felt that the effect was negligible, Boeck and Yater[3] found that carotene accounted for from 10 to 55% of the icteric index, while White[4] concluded that the influence was negligible except in marked cases of xanthemia accompanying diabetes.

The subject has been reinvestigated on 161 patients, 75 of whom were suffering from diabetes mellitus, the carotene being determined in terms of the icteric index (lipochrome index) and in mg. per 100 cc. of serum. In 43 diabetic cases on which icteric indices were determined carotenemia was found to account for an average of 45% of the corresponding icteric indices, the extremes being 19.3 and 70.5%. The average icteric index was 7.1. The average lipochrome index for the 75 diabetic patients was 3.4, corresponding to an average carotene content of 0.25 mg. per 100 cc. of blood.

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[1] Hess, A. F., and Myers, V. C., *J. Am. Med. Assn.*, 1919, **73**, 1743.

[2] Fiessinger, N., Walter, H., and Thierry, J. E., *Compt. rend. Soc. de Biol.*, 1928, **98**, 1299.

[3] Boeck, W. C., and Yater, W. M., *J. Lab. and Clin. Med.*, 1929, **14**, 1129.

[4] White, F. D., *J. Lab. and Clin. Med.*, 1931, **17**, 53.

The average lipochrome index for the 86 non-diabetic patients was 1.7 with an average of 0.11 mg. carotene per 100 cc. of blood. In 10 of these cases with icteric indices below 10, carotenemia was responsible for an average of 20.6% of the corresponding icteric indices, while in 21 cases with icteric indices between 10 and 100 it averaged 8.6% and in 6 cases with icteric indices above 100 the lipochrome indices averaged only 1.2% of the corresponding icteric indices.

From the above findings it would not appear that carotenemia alters the validity of the icteric index except in cases of marked carotenemia such as may be found in some diabetic patients, and then only if the icteric index is quite low.

7249 P

Effect of Dinitrophenol on the Oxygen Uptake of Rat Tissue.

EDWARD MUNTWYLER.

From the Department of Biochemistry, School of Medicine, Western Reserve University.

The observation by Cutting and Tainter[1] of a marked acceleration of the metabolism of animals injected with small quantities of dinitrophenol stimulated a study of the effect of the injection of the dinitrophenol on the oxygen uptake of rat tissues. Rats were injected with the sodium salt of dinitrophenol (40 mg. per kilo of body weight). After a period of from 30 to 40 minutes the animals were killed by a blow on the head and the oxygen uptake of the tissue determined as quickly as possible, employing the technique previously described.[2] The respiratory quotient was determined as described by Richardson.[3]

Table I presents the results which were obtained employing rat liver. It is seen that when phosphate Ringer's solution was employed, the oxygen uptake of rat liver slices obtained from the injected animals was essentially the same as that obtained with the

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[1] Cutting, W. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1268.

[2] Muntwyler, E., and Binns, D., *Am. J. Physiol.*, in press.

[3] Richardson, H. B., *Physiol. Rev.*, 1929, **9**, 61.

TABLE I.
Average Rate of O_2 Uptake of Rat Liver after Subcutaneous Injection of Dinitrophenol (40 mg. per kilo body weight).

No. of Animals	Oxygen Uptake in Minutes		Respiratory Quotient	Remarks
	60	120		
	Cu. mm. / mg. dry tissue			
3	9.2	15.8		Control —Phosphate Ringer's
5	8.4	15.4		Dinitrophenol—Average temperature increase 4.6° F.
3	11.9	21.8	0.807	Control —Glucose phosphate Ringer's
4	9.7	18.3	0.732	Dinitrophenol—Average temperature increase 4.9° F.

control. The oxygen uptake of the liver tissue obtained from injected animals, when suspended in phosphate Ringer's solution containing glucose, was slightly less than that of the control liver. The respiratory quotient of the liver tissue from injected animals was lower than that of the control liver.

Experiments are now in progress in which observations are being made on the oxygen uptake of other rat tissues and also experiments dealing with the effect of the dinitrophenol on the oxygen uptake of rat tissue *in vitro*.

7250 P

The Acid-Base Balance of the Blood in Migraine.*

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From the Department of Biochemistry, School of Medicine, Western Reserve University.

The present study was prompted by the findings of R. and S. Weissmann-Netter[1] who have made a series of observations on the carbon dioxide and the pH of the blood of patients with migraine. They found that the hydrogen ion concentration and the alkali reserve are normal in the periods of freedom from attacks but that a tendency to alkalosis develops 48 hours prior to an attack. They also reported that hyperventilation may result in an attack of migraine, probably through an excessive loss of carbon dioxide. As a result of the apparent success of controlling the migraine attacks by treatment with ketogenic diets,[2] it seemed desirable to reinvestigate the acid-base balance of the blood of individuals subject to migraine. To date 10 cases of migraine have been followed at intervals, the blood having been taken as early in the attack as possible, at the height of an attack, and immediately after cessation of the attack. It was found that the serum pH, CO_2 content, chloride and total base remained within normal limits, if one excepts 2 cases in which the pH was elevated slightly, due in all probability to the accompanying vomiting. It is probably of some significance that a fairly definite elevation of the blood cholesterol occurred in 50% of the cases.

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* Aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Weissmann-Netter, R., and Weissmann-Netter, S., *Compt. rend. Soc. de biol.*, 1925, **92**, 341.

7251 C

A Rapid Method for Analyzing Time-dilution Curves for Haemolytic Systems.

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From Washington Square College, New York University.

The time-dilution curve obtained for most haemolytic systems, which is described by the equation

$$kt = \frac{p}{p-1}\left\{ c^{\frac{p-1}{p}} - (c-x)^{\frac{p-1}{p}} \right\}$$

can be analyzed by a method already described (Ponder and Yeager[1]). The method is somewhat laborious and the analysis is more easily carried out as follows:

One requires a piece of cellophane, about 40 cm. square. The length of the right hand side is divided in such a way that the divisions represent 12.2, 24.4, 36.6, 48.8, 61.0, 73.2, 85.4, and 100% of that side. A series of lines are drawn on the cellophane joining each of these divisions to the left hand corner.

The time-dilution curve is drawn on a large scale in the usual way, and the asymptote (x) is marked with a horizontal line. Place the lower edge of the cellophane along the abscissa of the curve, and move it from right to left until the oblique line corresponding to the 100% division passes through the point at which the horizontal line corresponding to the asymptote cuts the ordinate of the curve. The oblique lines then cut the ordinate of the curve at a series of points. From the curve, read off the time corresponding to each of these points, add up the times, and divide by the time corresponding to the oblique line for 85.4%. Call the result S. The value of n(=1/p) is found by consulting Table I.

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[2] Barborka, C. J., *J. Am. Med. Assn.*, 1930, **95**, 1825.

[1] Ponder and Yeager, *Proc. Roy. Soc. B.*, 1930, **106**, 506.

TABLE I.

n	S	n	S
1.0	3.083	2.2	1.631
1.2	2.603	2.4	1.518
1.4	2.335	2.6	1.431
1.6	2.097	2.8	1.357
1.8	1.908	3.0	1.300
2.0	1.753		

In this way a time-dilution curve can be analyzed in less than a minute, and, if the curve is of the usual form, the value of n is obtained with an error no greater than ±0.05, for values not shown in the table can be found by interpolation. When large numbers of time-dilution curves are obtained in the course of an investigation, a rapid method of analysis is a great convenience, although it is often necessary to analyze the curves more carefully later on.

7252 C

Effect of Heat on Vitamin G Potency of Desiccated Yeast.

FRANKLIN C. BING AND DONALD G. REMP.

From the Department of Biochemistry, School of Medicine, Western Reserve University.

In the course of their investigations on the nutritive requirements of the chick Elvehjem, Kline, Keenan and Hart[1] found that about half of the vitamin G potency of desiccated yeast was destroyed by heating at 100°C. for 6 days. With the hope of securing evidence of the supposed duality of vitamin G, as postulated by Sure[2] and others, we have studied the effect of heat on both the growth-promoting and dermatitis-preventing activities of dried yeast. After preliminary desiccation the yeast was heated in an electric oven at either 105°C. or 150°C. for various lengths of time. The resulting products were fed, at a level of 1.5 gm. per mouse per week, to 3-weeks-old mice maintained on an otherwise G-deficient diet. The basal ration was prepared by mixing 200 gm. of vitamin-free casein, 265 gm. of cornstarch, 205 gm. of sucrose, 200 gm. of lard and 40 gm. of McCollum's salt mixture. Each animal received apart from the basal diet 2 drops of a tested cod liver oil and an ample amount of a vitamin B preparation. The latter was made from wheat germ by extraction with 93% alcohol, distillation of the extract *in vacuo*, and separation of the potent aqueous residue from an inert oil.

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[1] Elvehjem, C. A., Kline, O. L., Keenan, J. A., and Hart, E. B., *J. Biol. Chem.*, 1932, **99**, 309.

[2] Sure, B., Smith, M. E., and Kik, M. C., *Science*, 1931, **73**, 242.

The vitamin G activity of the yeast preparations, as measured by the growth response of the mice, is recorded in Fig. 1. No decrease in the vitamin G potency of yeast heated at 105° was observed. Further experiments with mice fed varying amounts of dried yeast, heated at 105° for 2 weeks, would be necessary to reveal whether a slight inactivation occurs. From the limited data available, however, it may be concluded that the destruction of vitamin G under these conditions is certainly not extensive. Moreover, Block and Farquhar,[3] who used the rat as their experimental animal, have recently reported that they observed no decrease in the growth-pro-

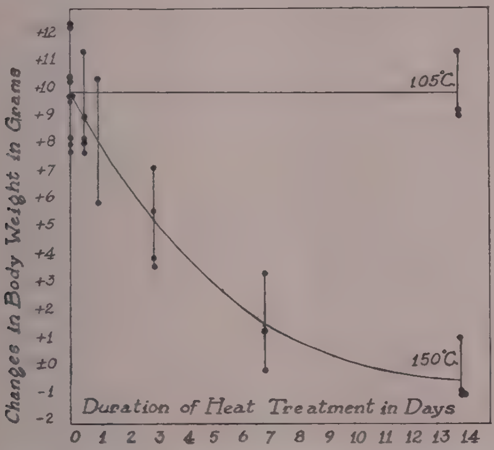

FIG. 1.—The Inactivation of Vitamin G by Heat.
Dots represent growth of mice in 6 weeks plotted against the length of time at which the desiccated yeast has been heated at either 105°C. or 150°C. The curves are drawn through the average value of each group.

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[3] Block, R. J., and Farquhar, L. B., *J. Biol. Chem.*, 1933, **103**, 643.

moting activity of yeast that had been heated at 100° for 2 or even 4 weeks.

Our data show further that the growth-promoting activity of yeast is slowly lost as a result of prolonged heating at 150°. There is a little vitamin G remaining even after one week of such treatment, but the vitamin is entirely destroyed in 2 weeks. Each of the 4 mice receiving the yeast that had been heated at 150° for 2 weeks developed typical skin lesions, as described by Bing and Mendel,[4] and all were dead in 7 weeks. At this time the mice in the other groups were still alive and their skins were in good condition. It appeared that the mice which had received sufficient vitamin to permit some growth had likewise received enough of the anti-dermatitis factor to meet their requirements, and the experiment was therefore discontinued.

As a guide to further experiments the slow rate of destruction of vitamin G at 150° is of special significance. If inactivation at the much lower temperature of 100° should occur, one could hardly expect to demonstrate it by biological assay except after unusually long periods of heat treatment.

From the results described herein it is concluded that the heating of previously desiccated yeast at 105°C. for 2 weeks probably does not affect its vitamin G content. Heating the yeast at 150°C. for the same length of time brings about a complete loss of activity; the resulting product neither permits growth nor prevents dermatitis on a vitamin G deficient diet. If there should prove to be separate factors for growth and dermatitis prevention in so-called vitamin G preparations they would thus appear to be destroyed at identical rates by heating in the dry condition.

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[4] Bing, F. C., and Mendel, L. B., *J. Nutrition*, 1929, **2**, 49.

7253 C

Metallic Glutamates in Nutritional Anemia.*†

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From the Department of Chemistry, New York State Psychiatric Institute and Hospital, New York.

Our knowledge of some of the many aspects of the problem of anemia has been enlarged recently by studies on the milk anemia of the rat. Thus from the work of the Wisconsin investigators the importance of Cu in Hb regeneration is now generally appreciated. Cu therapy, however, does not present any particular difficulty, while Fe deficiencies and Fe therapy still remain important problems in the clinic. For such investigations milk anemia is a convenient experimental approach. Although much work has been done there is still some uncertainty regarding the response of anemic rats to small doses of Fe.[1, 2] Certain investigators[3, 4] have reported that glutamic acid supplements Fe in nutritional anemia, but this has not been confirmed by others.[5]

The following experiments were carried out with about 300 rats in order to clarify some of the discrepancies in regard to the effect of Fe in anemia and to determine whether glutamic acid could be employed as an effective vehicle for the administration of minerals in secondary anemias.

Experimental. Most of the rats employed in the experiments were bred according to a modification of Elvehjem's technique.[6] The anemia producing diet was powdered whole milk (Klim, containing 1.8-2.0 p.p.m. of Cu and 14-17 p.p.m. of Fe) which was fed in the solid form. Hb estimations (Newcomer) were made weekly and red blood counts at frequent intervals. The inorganic Fe and Cu salts employed for supplements were of the highest purity; the

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* In cooperation with the Research Department of the Calco Chemical Co., Bound Brook, N. J.

† Part of this paper was presented before the Division of Biological Chemistry at the Spring Meeting of the American Chemical Society, Washington, D. C., 1933.

[1] Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

[2] Mitchell, H. S., and Miller, L., *J. Biol. Chem.*, 1931, **92**, 421.

[3] Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **93**, 39.

[4] Rider, T. H., *J. Biol. Chem.*, 1933, **100**, 243.

[5] Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1931, **93**, 197. Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1932, **97**, 115.

[6] Elvehjem, C. A., and Kemmerer, A. R., *J. Biol. Chem.*, 1931, **93**, 189.

ferrous glutamate was Cu-free and the glutamic acid contained less than 0.2 mg. Cu per 100 gm.

Some of the experiments with $FeCl_3$, ferrous glutamate and glutamic acid are summarized in Table I. It appears from this table that Hb regeneration is just as effective with ferrous glutamate (Group 1) as with ferric chloride (Group 2) and that addition of glutamic acid produced similar results (compare groups 2 and 3). When glutamic acid (Group 5) was added to supplements of Fe+Cu (Group 4) the hematopoietic response appeared to be slightly greater.

TABLE I.
Response of Anemic Rats to $FeCl_3$, Ferrous Glutamate and Glutamic Acid.

						Aver. Hb		
Group	Supplement	No. of Rats		Initial	2nd wk.	4th wk.	6th wk.	8th wk.
1	.25 mg. Fe as Ferrous Glutamate	5	a[1]	3.3	4.7	4.9	5.4	7.7
			b[2]	.21	.32	.37	.46	.74
2	.25 mg. Fe as $FeCl_3$	10	a	3.3	5.3	6.0	7.1	8.1
			b	.17	.33	.46	.63	.79
3	.25 mg. Fe ($FeCl_3$) + 70 mg. Glutamic Acid	14	a	3.2	4.7	5.6	6.5	8.0
			b	.20	.37	.50	.67	.88
4	.25 mg. Fe ($FeCl_3$) + .01 mg. Cu ($CuSO_4$)	7	a	3.9	6.7	9.5	11.6	13.7
			b	.33	.64	1.04	1.33	1.73
5	.25 mg. Fe ($FeCl_3$) .01 mg. Cu ($CuSO_4$) + 70 mg. Glutamic Acid	5	a	3.6	7.9	11.2	14.2	15.3
			b	.28	.72	1.13	1.48	1.75

[1] a = Hb in gm. per 100 cc. of blood.
[2] b = "Total" Hb in gm., *cf.* Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, XIII.

In view of this possible supplementary effect of glutamic acid when fed in conjunction with Fe and Cu, additional experiments were conducted. Groups of rats were fed the following combinations of supplements: 1. Mixtures of ferrous glutamate and copper glutamate at several levels of Fe and Cu. 2. Mixtures of ferrous glutamate and inorganic copper at several levels of Fe. 3. Mixtures of ferrous glutamate and inorganic Cu at several levels of Cu. 4. Mixtures of inorganic Fe and copper glutamate at several levels of Cu. 5. Copper glutamate at several levels of Cu. 6. Iron magnesium glutamate at several levels of Fe and Mg. 7. Iron magnesium glutamate plus glutamic acid. 8. Mixtures of Fe, Cu, Mg and Mn as (a) inorganic salts, (b) glutamates, (c) inorganic salts plus glutamic acid, (d) glutamates plus glutamic acid. Because of the similarity of the results, only the data from group 8 (a-d) will be presented in Table II.

TABLE II.
Response of Anemic Rats to Metallic Glutamates.

				Average Hb Values						
				Weeks						
Gr.	Supplement	No. of Rats		0	1	2	3	4	5	6
A	.25 mg. Fe as $FeCl_3$ .01 ’’ Cu ’’ $CuSO_4$ .30 ’’ Mg ’’ $MgCl_2$ .20 ’’ Mn ’’ $MnSO_4$	9	a[1]	2.8	5.6	7.0	8.4	9.6	12.3	12.7
			b[2]	.15	.37	.55	.72	.90	1.23	1.33
B	.25 mg. Fe .01 ’’ Cu .30 ’’ Mg .20 ’’ Mn glutamates (Bivalent)	10	a	3.0	5.1	6.8	8.3	9.8	11.6	12.0
			b	.16	.34	.51	.68	.86	1.08	1.19
C	.25 mg. Fe as $FeCl_3$ .01 ’’ Cu ’’ $CuSO_4$ .30 ’’ Mg ’’ $MgCl_2$ .20 ’’ Mn ’’ $MnSO_4$ + 67 ’’ Glutamic Acid	4	a	2.9	5.0	6.7	8.1	10.0	10.7	12.6
			b	.19	.36	.58	.79	1.05	1.22	1.49
D	.25 mg. Fe .01 ’’ Cu .30 ’’ Mg .20 ’’ Mn glutamates (Bivalent) 60 ’’ Glutamic Acid	4	a	3.8	4.6	6.4	7.8	9.1	10.0	12.0
			b	.25	.36	.57	.80	1.03	1.21	1.51

[1] Hb in gm. per 100 cc.
[2] ‘‘Total’’ Hb in gm., *cf.* Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, XIII.

It may be seen (Table II) that the glutamate compounds of Fe, Cu, Mg and Mn produced almost identical Hb regeneration and growth as equivalent amounts of these metals in inorganic form (See groups A and B). The addition of large amounts of glutamic acid (67 mg.) did not accelerate blood regeneration and growth (See groups C and D).

It should be noted that in all cases where Fe supplements only (Table I) are fed, there is a slow regeneration of Hb, which result is in accord with the findings of Mitchel and Miller.[2] On the other hand in practically every group reported in Table I some of the rats failed to survive the experimental period of 8 weeks, which is in agreement with the observations of the Wisconsin investigators.

Summary. The influence of glutamic acid in the remission of milk anemia in rats was investigated and the efficiency of this substance as a vehicle for the administration of metals as hematopoietic agents tested. The effect of inorganic salts and of metallic glutamates was essentially the same and the further addition of glutamic acid did not alter this result.

7254 C

Pigmentation and Growth Rate in Gray Breast Feathers of Adult Mallard Drakes.

R. GEORGE JAAP. (Introduced by L. J. Cole.)

*From the Department of Genetics (No. 171), University of Wisconsin.**

Before the physiology of sexual dimorphism in birds can be completely understood the normal change from the male winter plumage to the female-like summer plumage in the Mallard drake must be explained. This report embodies certain facts regarding feather pigmentation and growth rate in Mallard drakes which must be considered in future studies of the physiological basis for this "eclipse" or "summer plumage" change of the adult male duck.

The males used in these experiments are semi-domesticated Mallards, the majority of which are genetically "dark-phase mallard" *(M Li)*.[1] These drakes are very uniform in breeding, and in adult body weight, between 980 and 1160 gm. The males are left in outside yards until they are to be used in an experiment, when they are brought indoors and kept in artificially heated and lighted pens, the hours of illumination being approximately the same in all trials. At the beginning of the experiment, 50 to 60 feathers are plucked from the middle of the ventral surface on either side of the keel, the plucked area being in the same location on each drake. All trials

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[1] Jaap, R. G., *J. Her.*, 1933, **24**, 467.

were conducted between the months of September and the following April. At any time within this period the growing feathers normally show no detectable variation in pigmentation or growth rate.

The depth of color in the growing gray breast feathers may be increased artificially by 2 different treatments, oral administration of desiccated sheep thyroids (Armour & Co. preparation, 0.2% iodine), or subcutaneous injection of an extract of the urine of young men, prepared according to the method of Funk, Harrow and Lewja[2] for male hormone. The thyroid increases the pigmentation and the growth rate of the feathers, and the urine extract augments the pigment increase stimulated by the thyroid. The increase of pigmentation in the partially-grown gray breast feathers is recorded in the tables by direct comparison of the normal and modified feathers of each drake. Each plus symbol indicates a definite intensification of the melanic pigment. Feathers with 5 plus symbols, in addition to being very dark in color, have a definite edging of light brown and in this respect resemble the feathers of the eclipse (summer) plumage. Each drake was killed at the termination of the experiment and, in parts B and C of Table I, a certain number of growing feathers were removed for measurement. At

TABLE I.

Drake No.	Exp. started	Daily thyroid dose gm. per kilo body wt.	Mean length of feathers Cm. P.E.	Wt. of testes Gm.	Pigmentation change
A.—Treated six weeks					
WB22	Dec. 29	--------		4.08	0
357	” ”	.25		9.98	0
774	” ”	.50		18.13	+
WB14	Feb. 9	--------		7.72	0
WB13	” ”	.50		32.52	++
6938	” ”	1.00		67.52	+++
B.—Treated 18 days			(30 feathers)		
2W	Sept. 1	--------	2.26 ± .05		0
2N	” ”	.50	2.87 ± .08		+
2.1	” ”	1.50	3.06 ± .03		+++
2R	” ”	3.00	3.07 ± .04		+++++
C.—Treated 17 days			(10 feathers)		
79B	Oct. 5	--------	2.03 ± .10	2	0
51C2	” ”	.1	2.66 ± .04	6	0
2.6	” ”	.2	2.83 ± .04	3.5[1]	+
49C2	” ”	.4	2.70 ± .10	1.5[2]	0
2.5	” ”	.6	2.84 ± .05	7	++
49G2	” ”	.8	2.74 ± .08	18	++
46T2	” ”	1.0	2.79 ± .05	15	+++
24C	” ”	2.0	2.72 ± .03	33	++++

[1] Left testis, 0.5 gm.; right testis, 3.0 gm.

[2] Testes dark color, microscopically showing pronounced regression.

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[2] Funk, C., Harrow, B., and Lewja, A., *Am. J. Physiol.*, 1930, **92**, 440.

this time the testes were weighed, and pieces from these and from the thyroids saved for histological study.

Table I shows that levels of desiccated thyroid above 0.2 gm. per kilo body weight increase the pigmentation of the gray breast feathers of the Mallard drake. The weight and spermatogenic activity of the testes of treated animals increase over those of the non-treated controls in almost direct proportion to the amount of thyroid fed. It will be noted that the testis weights of non-treated birds vary according to the time of the year in which the trial was concluded. This phenomenon has been recorded by many previous investigators.

Growth rate, as measured by the mean lengths of the immature feathers plucked, shows an increase on the lower thyroid levels, but fails to respond further on the higher doses. A marked and significant increase in growth rate is obtained on a 0.1 gm. level of thyroid, without any quantitative change in pigmentation of the feathers. Thyroid, therefore, has two distinct and separable effects, (1) increasing the growth rate and (2) increasing the pigmentation. Lillie and Juhn[3] found in the Brown Leghorn fowl that the feathers in certain sections of the plumage grow more rapidly than in others. Also, they have shown that such feathers require a higher concentration of hormone to increase their pigmentation than those in areas of lower growth rate, demonstrating that more rapidly growing feathers have a higher threshold for pigmentation. Since thyroid feeding increases the growth rate of the feathers in the breast area of the Mallard drake, it is assumed that levels of 0.1 to 0.2 gm. of thyroid are not sufficiently high to overcome this pigmentation threshold. A higher dose (0.6 gm.) fails to stimulate further increase in the growth rate and intensifies the color of the feathers by overcoming the threshold for pigmentation. The growth rate remains more or less constant on levels over 0.6 gm., while the increase in feather pigmentation is then roughly proportional to the amount of thyroid administered.

There is evidence from the foregoing experiments that the size and corresponding activity of the testes are, in some manner, related to increased pigmentation of the feather. This association occurs under normal conditions, for the drake exhibits the ability to produce the more deeply pigmented summer plumage feathers at a time when the testes are large and active. While there is considerable disagreement in the literature, the majority of authors find that complete gonadectomy inhibits the summer plumage change.[4] Three completely castrated drakes have been given 0.25, 0.5 and 0.75 gm. levels of thyroid respectively. None of these has produced any feathers darker than normal, while non-castrated drakes under similar treatment show an increase in feather pigmentation on the 2 higher levels. It appears, therefore, that there may be a quantitative relationship between testis and the thyroid which increases the pigment-forming action of the latter. A trial was conducted in an attempt to further modify the pigmentation by increasing the "male hormone" concentration in both normal and thyroid-fed drakes. This trial was then repeated with higher levels of thyroid. The results of both trials on non-castrated drakes are presented in Table II.

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[3] Lillie, F. R., and Juhn, Mary, *Physiol. Zool.*, 1932, **5**, 124.

TABLE II.

Drake No.	Exp. started	Daily treatment Thyroid gm.	Daily treatment Urine extract cc.*	Wt. of testes gm.	Pigmentation change
A.—Treated 26 days					
453	Nov. 12	-------	-------	7.02	0
426	” ”	-------	1.0	4.25	0
421	” ”	.5	-------	4.92	+
452	” ”	.5	1.0	34.08	++++
B.—Treated 30 days					
252	Dec. 23	-------	-------	5.25	0
305	” ”	-------	1.0	23.78	0
313	” ”	1.0	-------	25.78	+++
308	” ”	1.0	1.0	51.62	+++++

* One cubic centimeter of extract is equivalent to 200 cc. of urine.

It will be noted that, although this urine extract when administered alone has no detectable influence on the feathers, it produced a much greater pigmentation in combination with thyroid than did the thyroid substance itself. An even more surprising result is that in both of these trials the testis is larger and more active when both thyroid and urine extract are administered together. It is certain that the testis secretion in some manner augments the pigment deposition initiated by the thyroid substance. The cause of the increased testis activity under the combined treatment is being investigated further.

Since Tallent[5] has shown that injection of an anterior pituitary suspension produces feathers similar to those of the eclipse plumage, we may illustrate what appears to be the essential glandular interaction in the production of the intensely pigmented summer plumage of the gray breast area by the following diagram:

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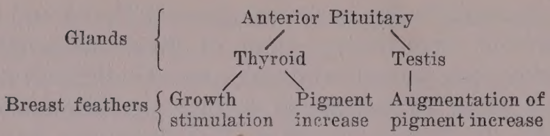
[4] Allen, E., *Sex and Internal Secretions*, Williams & Wilkins Co., 1932, 584.

[5] Tallent, Violet K., *Nature*, 1931, **128**, 672.



7255 C

Growth of Bone Shafts in the Human Fetus.

ROBERT ROBB AND JOHN CLARKE. (Introduced by Frank P. Knowlton.)

From Syracuse University College of Medicine.

If individual bones differ in their rates of growth and if each of the major long bones is the product of 3 to 8 centers of ossification one may feel surprised at the linear relationships reported by Scammon and Calkins[1] between diverse *external* dimensions of the human fetus. We need assurance that the simplicity of these gross observations does not mask a greater complexity in the constituent tissues.

In this fetal series we report the lengths of the bone shafts alone, before the appearance of osseous epiphyses, from dried preparations in the Department of Embryology. The observations are well distributed through fetal life, their variability is small, and the error of measurement is less than 2%, but we do not know what change took place in drying.

The rectilinear relationships in Fig. 1 demonstrate our confirmation, by a different technique, of Scammon and Calkins'[1] belief that the relative growth of human fetal long bones is approximately linear. This interpretation applies also to fetal skull growth in the horse[2] but fetal limb growth in the horse (to be published) required a second degree equation. We have also replotted the data of Schmalhousen and Stepanowa[3] concerning bone growth in the chick embryo. Here we find that lengths, as well as weights are interrelated as power functions and thus exhibit growth partition.[4, 5] The chick humerus length is a function of femur length with exponent 0.87, and approximately the same exponent, 0.88 describes

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[1] Scammon, R. E., and Calkins, L. A., Growth in the Fetal Period. 1929. U. of Minn. Press.

[2] Robb, R. C., *Proc. Sixth Internat. Cong. Gen.*, 1932, **2**, 166.

[3] Schmalhousen, I., and Stepanowa, J., *Arch. Entw. d. Organ.*, 1926, **108**, 721.

[4] Huxley, J. S., Problems of Relative Growth, 1932. Dial Press, New York.

[5] Robb, R. C., *Brit. J. Exp. Biol.*, 1929, **6**, 311.

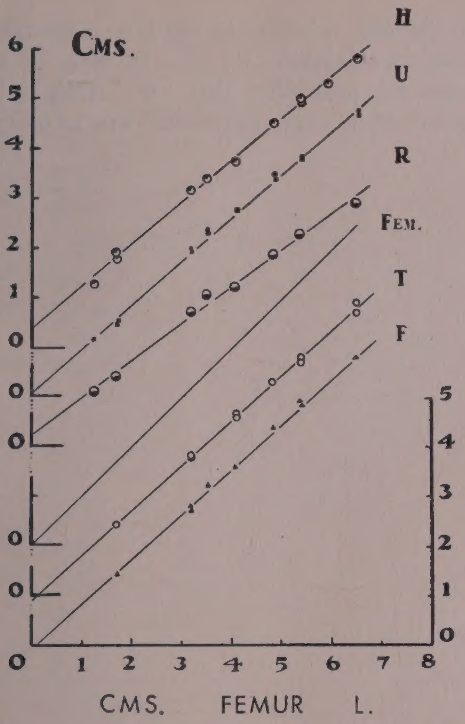

FIG. 1.

Lengths of bone shafts (diaphyses only) plotted against that of the femur in the human fetus. H—humerus, U—ulna, R—radius, Fem.—femur itself, T—tibia, F—fibula.

TABLE I.

Dimensions of Human Fetal Bone Shafts.

	Human Fetus							
	I	II	III	IV	V	VI	VII	VIII
CM. LENGTH								
Crown-Heel	9.0	13.0	18.0	22.	25.	30.	32.	37.5
Humerus, Right	1.29	1.80	3.19	3.40	3.81	4.56	5.04	5.83
Left	1.27	1.90	3.19	3.42	3.75	4.56	5.04	5.82
Ulna, Right	1.16	1.49	2.95	3.37	3.78	4.43	4.85	5.80
Left	....	1.50	2.98	3.38	3.78	4.49	4.85	5.80
Radius, Right	1.1	1.40	2.70	3.05	3.20	3.87	4.28	4.87
Left								
Femur, Right	1.29	1.71	3.20	3.52	4.10	4.84	5.40	6.50
Left	1.25	1.70	3.18	3.55	4.10	4.88	5.40	6.60
Tibia, Right	....	1.40	2.80	3.23	3.66	4.30	4.70	5.70
Left	....	1.40	2.70	3.21	3.59	4.31	4.80	5.90
Fibula, Right	....	1.40	2.75	3.20	3.60	4.37	4.88	5.80
Left	....	1.40	2.80	3.19	3.60	4.35	4.85	5.80
INDEX								
Humero-Femoral	1.01	1.08	1.00	0.96	0.92	0.92	0.93	0.89

radius or ulna growth, whereas in the hind limb the exponent for tibia or for tarso-metatarsus is 1.05. In view of these facts we cannot eliminate the possibility that our "straight lines" are but segments of power curves with exponents close to unity.